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The Pathogenesis of Bisphosphonate Related Osteonecrosis of the Jaw: So Many Hypotheses so Few Data

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29 **Abstract**

30 Bisphosphonate-related osteonecrosis of the jaw (BRONJ) has generated great interest in the
31 medical and research communities yet remains an enigma given its unknown pathogenesis. The
32 goal of this review is to summarize the various proposed hypotheses underlying BRONJ. While a
33 role of the oral mucosa has been proposed, the bone is likely the primary tissue of interest for
34 BRONJ. The most popular BRONJ hypothesis, manifestation of necrotic bone resulting from
35 bisphosphonate-induced remodeling suppression, is supported mostly by indirect evidence
36 although recent data has shown bisphosphonates significantly reduce remodeling in the jaw.
37 Remodeling suppression would be expected, and has been shown, to allow accumulation of non-
38 viable osteocytes while a more direct cytotoxic effect of bisphosphonates on osteocytes has also
39 been proposed. Bisphosphonates have anti-angiogenic effects, leading to speculation that this
40 could contribute to the BRONJ pathogenesis. Compromised angiogenesis would most likely be
41 involved in post-intervention healing although other aspects of the vasculature (e.g. blood flow)
42 could contribute to BRONJ. Despite infection being present in many BRONJ patients, there is no
43 clear evidence as to whether infection is a primary or secondary event in the pathophysiology. In
44 addition to these main factors proposed in the pathogenesis, numerous co-factors associated
45 with BRONJ (e.g. diabetes, smoking, dental extraction, concurrent medications), could interact
46 with bisphosphonates and affect remodeling, angiogenesis/blood flow, and/or infection. As our
47 lack of knowledge concerning BRONJ pathogenesis is due to a lack of data, it is only through the
48 initiation of hypothesis-driven studies that significant progress will be made to understand this
49 serious and debilitating condition.

50

51 **Key words:** Osteonecrosis of the jaw, bisphosphonates, bone remodeling, osteocytes,
52 angiogenesis

53

54 **Introduction**

55 Bisphosphonate-related osteonecrosis of the jaw (BRONJ) has become one of the most
56 prominent enigmas not only in the dental community, but in the field of skeletal biology as a
57 whole. Confusion surrounding BRONJ exists for several reasons, including a lack of
58 understanding about how and why this condition manifests ¹. Since brought to light in 2003/2004
59 ²⁻⁵, well over 400 manuscripts have been published concerning BRONJ. Despite this large
60 volume of work there remain few data, yet many hypotheses, concerning the underlying
61 pathophysiology. The goal of this review is to summarize some of the various proposed
62 hypotheses for the pathophysiology of BRONJ.

63

64 **The starting point for BRONJ: Bone or soft tissue?**

65 As its name implies, BRONJ is often assumed to be primarily a bone condition. The hallmark of
66 BRONJ is the existence of exposed bone with the majority of cases manifesting following dental
67 intervention ¹. As epithelialization is an essential step in post-intervention wound healing ^{6,7}, it
68 has been hypothesized that the soft tissue of the oral mucosa could play a significant role in
69 BRONJ. Specifically, it has been proposed that bisphosphonates, which accumulate in the bone,
70 have direct toxic effects on the oral epithelium and inhibit normal healing of soft tissue lesions
71 caused either by dental intervention or some other trauma ^{8,9}. The failure of soft tissue to heal
72 would result in exposure of the bone, which then becomes necrotic. Although dental extraction is
73 a significant risk factor for BRONJ ¹⁰, the condition does occur in the absence of dental
74 intervention ¹.

75 The effect of localized high concentration on the oral mucosa is most clearly illustrated by
76 the case report of stomatitis in a patient who held their bisphosphonate medication in their mouth
77 ¹¹. However, use of bisphosphonates in gel form for treatment of periodontal lesions, although
78 not widely studied and only used short-term, likely present the oral mucosa with high local
79 concentrations and have not shown any adverse effects ¹². Beyond this, however, little is known
80 about bisphosphonates and the oral mucosa.

81 One key unknown for the hypothesis of soft-tissue toxicity is whether the oral mucosa,
82 comprised of epithelial and vascular tissue, is exposed to sufficient bisphosphonate levels in vivo
83 to disrupt its normal physiology. Since bisphosphonates only accumulate in the bone ¹³, a
84 scenario would most likely have to exist in which large amounts of drug are liberated either all at
85 once, or at sufficiently high concentrations over a prolonged duration. It has been suggested that
86 this would occur during dental intervention due to physical disruption of the bone ^{8,9}, although this
87 has not been assessed and would not explain the occurrence of BRONJ in the absence of dental
88 procedures. Alternatively, sufficient concentrations of bisphosphonates in the saliva, or gingival
89 crevicular fluid could expose the oral mucosa to high levels of drug. Whether or not the several
90 BRONJ risk factors, such as diabetes, smoking, and concurrent medications, play a role in
91 compromising the oral mucosa is also unclear. Thus, while the hypothesis remains intriguing and
92 is worth further study, the skeleton seems most likely to serve as the central factor for initiation of
93 BRONJ.

94

95 **Bone cells and BRONJ: Where to focus attention**

96 The physiological effects of bisphosphonates on bone cells - osteoblasts, osteoclasts, and
97 osteocytes - have recently been expertly reviewed ^{14,15}. Osteoclasts (**Figure 1a**) are the main
98 cellular target of bisphosphonates ¹⁶. Specifically, through disruption of intracellular pathways,
99 bisphosphonates suppress osteoclast-mediated bone remodeling. As remodeling plays a vital
100 role in tissue renewal and bone healing, bisphosphonate-induced remodeling suppression
101 imparts significant effects on various tissue-level properties ¹⁷⁻²². The effects of bisphosphonates
102 on osteocytes (**Figure 1b**), the most abundant of the bone cells, are less clear and more
103 controversial. Evidence exists for both direct and indirect effects, most of which are centered
104 around the viability and integrity of these cells and their environment ^{20,23-26}. Osteoblasts
105 (**Figure 1c**) appear to be the least affected of the bone cells. . While systemic bone formation is
106 reduced in the presence of bisphosphonates, this is primarily an indirect consequence of
107 remodeling suppression and the coupling between resorption and formation. At the level of the
108 individual basic multicellular unit osteoblast activity appears unaffected ^{27,28}. Reports from small

109 animal models suggest that bisphosphonates may suppress osteoblastic bone formation directly
110 on those surface undergoing bone formation without prior resorption (i.e .formation modeling)²⁹,
111 although large animal models do not show a similar suppressive effect on periosteal surfaces³⁰⁻
112 ³². Collectively, this evidence points towards the osteoclasts and/or osteocytes as the main cells
113 of interest for BRONJ pathogenesis.

114

115 **Remodeling suppression and BRONJ: The basic premise of the most popular hypothesis**

116 Nearly every report and review of BRONJ points to bisphosphonate-induced remodeling
117 suppression as a likely mechanism. The basic premise of this hypothesis is that the jaw has a
118 high remodeling rate and bisphosphonates suppress remodeling. There is no debate about the
119 latter as this is the principal mechanism of action of the bisphosphonates^{14, 15}. It is also clear that
120 remodeling, specifically within the intracortical envelope, is considerably higher in the jaw
121 compared to other skeletal sites. As opposed to remodeling that occurs on bone surfaces,
122 intracortical (osteonal) remodeling occurs within cortical bone of humans and many large animals
123 ³³. In humans, intracortical remodeling rates of the jaw are 10-20 times higher than within the
124 cortex of the iliac crest^{34, 35}. Animal studies support the limited human data, consistently showing
125 that remodeling rates in the jaw are significantly higher than in the long bones^{36, 37}. The BRONJ
126 hypothesis thus follows the idea that since remodeling is high in the jaw, and bisphosphonates
127 suppress remodeling, this likely plays a role in the pathophysiology of BRONJ.

128 Bisphosphonate effects on intracortical bone remodeling of the mandible have only
129 recently been documented²⁰. Following three years of daily treatment with doses of oral
130 alendronate that were either equivalent to the clinical dose for osteoporosis, or 5x higher, the
131 level of intracortical remodeling was histologically assessed in skeletally mature beagle dogs.
132 Consistent with previous work by others, untreated animals had mandible remodeling rates,
133 specifically within the alveolar region, that were >10-fold higher than within long bones. Daily oral
134 alendronate significantly suppressed intracortical bone formation rate of the mandible compared
135 to vehicle, mainly due to suppression in the alveolar bone region (**Figure 2**)²⁰. These data
136 represent clear evidence of bisphosphonate-induced turnover suppression in the jaw and

137 although consistent with both components of the remodeling-suppression hypothesis for BRONJ
138 they do not establish a clear cause-effect relationship.

139 The incidence of BRONJ is significantly higher in cancer patients compared to those
140 treated for osteoporosis ¹ suggesting differences should exist in the degree of remodeling
141 suppression between these two populations. Compared to treatment regimens used for
142 osteoporosis, treatment regimens in cancer patients use bisphosphonates with higher potency
143 and binding affinity (zoledronate and pamidronate) and involve both higher doses and more
144 frequent dosing schedules. Differences in potencies and binding affinities among the
145 bisphosphonates are known to affect the degree of remodeling suppression ¹⁴, and both
146 treatment dose and duration are associated with BRONJ ^{10, 38-40}. Thus, it is reasonable to
147 hypothesize that cancer patients may experience a greater remodeling suppression within the
148 jaw, although such measurements have never been made even in pre-clinical models.

149 If remodeling suppression is part of the pathophysiology of BRONJ, one might expect it to
150 occur during the course of treatment with other anti-catabolic therapies. Although no BRONJ
151 cases have been reported in patients treated with other anti-remodeling agents (hormone
152 replacement therapy, selective estrogen receptor modulators, and calcitonin), these agents
153 typically do not suppress turnover by more than 50%. Denosumab, a monoclonal anti-RANKL
154 antibody currently in clinical trials for both osteoporosis and cancer populations, has been shown
155 to suppress remodeling an equal or greater extent than bisphosphonates ^{41, 42}. Although no
156 cases of BRONJ have been reported in patients treated with denosumab, increases in the
157 number of treated patients, as well as the duration of treatment, will help to answer questions
158 surrounding the role of remodeling suppression in BRONJ.

159 Individuals with genetic mutations affecting osteoclast activity provide a means of
160 studying the effects of significant levels of remodeling suppression ⁴³. Several of these genetic
161 conditions have been reported to produce BRONJ-like symptoms ^{44, 45}, supporting the idea of
162 remodeling-suppression in the pathophysiology. For example, patients with inactivating
163 mutations in the chloride channel 7 gene have autosomal dominant osteopetrosis (ADO), a
164 condition in which osteoclast resorption is significantly compromised ^{45, 46}. Jaw osteomyelitis was

165 noted in 13% of patients with ADO, compared to a complete absence of osteomyelitis in the
166 control population ⁴⁶. Interestingly, 5 of the ADO patients (8%) had draining fistulas and/or
167 obvious bony destruction resulting in visible defects in the jaw or palate, a similar clinical
168 presentation to that of BRONJ ⁴⁷. Patients with a different genetic condition, pyknodysostosis, an
169 autosomal recessive mutation in the cathepsin-K gene which inhibits osteoclast activity, have also
170 been shown to develop exposed bone in the oral cavity ^{48, 49}.

171 Perhaps the most intriguing reports supporting the remodeling suppression hypothesis of
172 BRONJ concern resolution of the condition subsequent to treatment with agents that stimulate
173 remodeling. In three separate case reports, patients with confirmed BRONJ were treated with
174 teriparatide (recombinant human parathyroid hormone (1-34)), an FDA approved agent for
175 treating post-menopausal osteoporosis which acts through stimulation of bone remodeling ⁵⁰⁻⁵².
176 While each of these cases involved numerous other interventions (including cessation of
177 bisphosphonate treatment, debridement, and anti-bacterial washes), the resolution of BRONJ
178 came only after introduction of teriparatide treatment.

179 The strongest challenge to the remodeling suppression hypothesis comes from children
180 with osteogenesis imperfecta. These patients are routinely treated with high doses of
181 bisphosphonates and to date there have been no reports of BRONJ ^{53, 54}. It is unclear if or why
182 bisphosphonates differentially affect remodeling in the jaw of young and old subjects.

183 An important aspect of the remodeling suppression hypothesis is that much of the focus
184 has been on the pre-existing bone which may not be the true site of interest. Similar to fracture
185 healing, after dental extraction the socket fills with woven bone which over time is remodeled into
186 lamellar bone ^{6, 7, 55}. The fracture healing literature clearly shows woven bone formation is not
187 compromised in the presence of bisphosphonates, yet remodeling of this callus is significantly
188 delayed ^{22, 56-58}. Thus, it may be that bone formed early during oral wound healing is not
189 remodeled in a timely fashion and this in turn develops into BRONJ. Although some investigation
190 has occurred looking at extractions in the presence of bisphosphonates ^{59, 60}, these studies have
191 focused on the preservation of the alveolar bone structure. There exist no data to describe how
192 bisphosphonates affect bone that is formed post-extraction or how it is remodeled over time.

193 The hypothesis of remodeling suppression as a factor in the pathophysiology of BRONJ
194 makes sense and is supported by some data. Yet a key unanswered question is how the
195 suppression of remodeling, even at a site with high turnover, results in necrotic bone and why this
196 seems specific to the high doses of intravenous bisphosphonates.

197

198 **Remodeling suppression and BRONJ: Focus on the osteocyte**

199 There exists limited histological assessment of BRONJ tissue, yet that which exists almost
200 universally notes the presence of empty lacunae – void of their resident osteocytes. Osteocytes,
201 the most abundant bone cells, form an intricate communication network throughout the
202 mineralized matrix (**Figure 3**) and play a key role in skeletal physiology^{61, 62}. While generally
203 considered a long-lived cell, the lifespan of the osteocyte is finite and therefore over time these
204 cells undergo natural death⁶³⁻⁶⁵. Under normal physiological conditions, loss of osteocytes and
205 the associated changes to tissue can likely be held in check by bone remodeling⁶⁴. Yet as
206 bisphosphonates suppress remodeling, regions of non-viable osteocytes would be expected to
207 accumulate.

208 Focal loss of viable bone matrix has been documented in a pre-clinical animal model.
209 Following three years of treatment with oral bisphosphonates, mandibles of beagle dogs
210 contained significant regions of non-viable bone matrix²⁰. Using en bloc basic fuchsin staining
211 which fills all voids within the matrix (microdamage, Haversian canals, osteocyte lacunae and
212 canaliculi^{66, 67}), regions of non-viable bone matrix were identified by the absence of stain
213 suggesting the osteocyte network had filled with mineral (**Figure 4**). Non-viable matrix was noted
214 in a fraction of bisphosphonate-treated animals (~30%), most often in the alveolar portion of the
215 bone, yet was not observed in any control animals²⁰. Using this same basic fuchsin technique,
216 regions of matrix necrosis can be observed in pathological samples from BRONJ patients (**Figure**
217 **5**). Previous studies on samples from patients with BRONJ, using more standard methods of
218 histological evaluation with hematoxylin and eosin staining, have also observed areas of bone
219 tissue with empty lacuna interspersed among areas of vital bone⁶⁸. Although it remains unclear if

220 or how these areas of focal matrix necrosis play into BRONJ ⁶⁹ these findings suggest the
221 osteocyte could have a central role in the pathophysiology.

222 The accumulation of non-viable osteocytes in association with bisphosphonate treatment
223 could manifest through indirect or direct mechanisms. As outlined above, osteocyte death is a
224 natural process and through suppression of remodeling it would be expected that regions with
225 non-viable cells would be more prevalent. This accumulation would have little to do with
226 bisphosphonates, per se, but rather would be a result of suppressed remodeling. If this
227 hypothesis is correct, it would be expected that other anti-remodeling agents, or other conditions
228 which result in remodeling suppression, would also result in an accumulation of non-viable bone,
229 and that it would be dose- or potency-dependent. Additionally, it would be expected that regions
230 of non-viable bone would not be confined to the mandible but would be present throughout the
231 skeleton.

232 An alternate hypothesis for the accumulation of non-viable osteocytes with
233 bisphosphonates is through a direct effect of these drugs on the osteocytes ^{9,69}. It is well
234 accepted that bisphosphonates become embedded in the skeleton and therefore accumulate
235 over time ^{70,71}. It has recently been demonstrated that systemically administered
236 bisphosphonates have access to, and become embedded in, the osteocyte lacunae ⁷². As such,
237 it is possible that osteocytes could be exposed to high concentrations of bisphosphonates over
238 time which in turn could affect cell viability.

239 The effects of bisphosphonates on osteoblast/osteocyte viability have been
240 predominantly investigated in vitro. Through connexin (Cx)-43 hemichannel transduction of
241 extracellular signal regulated kinases (ERKs), low concentrations of bisphosphonates have been
242 shown to suppress osteocyte apoptosis through maintenance of cellular connections ^{26, 73-75}.
243 These results have translated well to in vivo models where bisphosphonates have also been
244 shown to suppress prednisone-induced ²⁶ and mechanically-induced ²⁵ osteocyte apoptosis.
245 However, the anti-apoptotic effects in vitro appear to be dose-dependent such that higher
246 concentrations increase osteocyte apoptosis ²⁴. This establishes a plausible scenario where
247 osteocytes are initially exposed to low levels of bisphosphonates which prolong osteocyte

248 longevity yet with continued treatment, particularly at high doses, concentrations of drug
249 accumulate near the osteocyte which results in cell death.

250 Whether these direct or indirect pathways connecting bisphosphonates to loss of
251 osteocyte viability play a role in BRONJ is unclear. Of the two, the direct pathway is more
252 consistent with numerous clinical aspects of the condition. BRONJ is more prominent in patients
253 treated with high-doses of intravenous pamidronate or zoledronate, as compared to those treated
254 at lower doses for osteoporosis¹. Intravenous administration results in a higher skeletal
255 accumulation as compared to oral administration¹³, while pamidronate and zoledronate have the
256 highest mineral binding affinities among all of the bisphosphonates^{76,77}. The increased risk of
257 BRONJ associated with treatment duration is also consistent with the accumulation of drug over
258 time.

259 Given the central role of osteocytes in the regulation of the skeleton, understanding how
260 pharmacological agents affect their physiology is essential. The effects of bisphosphonates on
261 osteocytes are only now beginning to be understood. Despite indirect evidence that the loss of
262 osteocyte viability could play a role in the pathophysiology of BRONJ, the paucity of data results
263 in this remaining a hypothesis.

264

265 **BRONJ and vasculature: The anti-angiogenic effects of bisphosphonates.**

266 Prior to the emergence of BRONJ, much of what was known concerning osteonecrosis centered
267 on two conditions which manifest due to disruptions in vasculature. Avascular necrosis of the hip
268 occurs secondary to disruption of the vasculature⁷⁸. Similarly, osteoradionecrosis, most
269 prominently of the jaw, occurs following radiation-induced disruption of the vasculature^{68, 79, 80}.
270 The existence of these conditions, and the clear role of disrupted vasculature in their
271 pathophysiology, has led to the hypothesis that the vasculature plays a key role in
272 pathophysiology of BRONJ.

273 A role of the vasculature in BRONJ has been mostly fueled by studies showing anti-
274 angiogenic properties of bisphosphonates. Indeed, bisphosphonates are emerging as a potential
275 means of suppressing angiogenesis associated with tumor growth^{81,82}. Numerous studies have

276 documented anti-angiogenic effects of bisphosphonates in vitro while a smaller number have
277 shown similar effects in vivo. The latter include suppression of angiogenesis in subcutaneously
278 implanted tissue chambers⁸³, reduced testosterone-induced prostate tissue re-vascularization
279 following castration⁸⁴, and significant reductions in marrow vessel number of iliac crest biopsies
280 after six months of clodronate treatment for Paget's disease⁸⁴. Conversely, early in vivo studies
281 with high doses of bisphosphonates did not document altered vascular invasion near the growth
282 plates⁸⁵. There have been no systematic studies assessing the vascular pattern in BRONJ.
283 Qualitatively, the vasculature has been reported to be intact in a series of BRONJ cases⁸⁶ while
284 a separate series reported 'vessel obliteration' in some BRONJ specimens^{68,87}. In the dog
285 model of matrix necrosis the vasculature appears to be patent and intact even in regions devoid
286 of viable osteocytes²⁰.

287 Recently, two cases of exposed bone in the mandible, similar in nature to BRONJ, have
288 been reported in cancer patients treated with Bevacizumab, a recombinant human monoclonal
289 antibody that binds to vascular endothelial growth factor (VEGF) and inhibits angiogenesis⁸⁸.
290 These patients were not treated with bisphosphonates and did not undergo any dental
291 intervention. This provides the strongest evidence to date suggesting a role of the vasculature in
292 BRONJ.

293 Reduced angiogenesis within bone would actually be expected to occur with
294 bisphosphonates, due to a suppression of remodeling. Each remodeling unit receives its
295 nutrients by a vessel⁸⁹; therefore bone remodeling and angiogenesis are intimately linked.
296 Bisphosphonate-induced reductions in remodeling should be associated with reduced
297 angiogenesis yet the reduction would be a secondary effect. It remains unclear if this has
298 relevance to BRONJ.

299 Perhaps the most intriguing role of altered angiogenesis with bisphosphonates may be
300 related to wound healing⁹⁰. Following tooth extraction, a major precipitating event in BRONJ¹⁰,
301 the extraction site undergoes a well-defined series of healing steps which include an initial clot
302 formation, conversion of clot to granulation tissue, formation of connective tissue and pre-
303 osseous tissue, and finally filling of the extraction socket with bone^{7,91-93}. Disruption of this

304 normal process at any stage, particularly the formation of the provisional matrix that occurs early
305 during treatment, could compromise the entire process⁹⁰. Furthermore, disruption of the
306 remodeling of this extraction site by osteoclasts, which normally occurs via an accelerated rate of
307 modeling and remodeling^{55, 93}, may potentially play into the lack of healing that is a prominent
308 feature of BRONJ. The sole evidence on this topic comes from an in vivo study in mice which
309 showed that bisphosphonates did not affect angiogenesis associated with endochondral
310 ossification, a process that is similar to that which occurs with skeletal wound healing⁹⁴.

311 Another aspect related to vasculature, but not as routinely discussed in the BRONJ
312 literature as angiogenesis, is potential effects of bisphosphonates on blood flow⁹⁰. Tissue blood
313 flow is directly proportional to its metabolic activity with the bone receiving ~4-7% of total cardiac
314 output at rest⁹⁵ compared to ~17% for skeletal muscle⁹⁶. Blood flow distribution throughout the
315 skeleton is heterogeneous and varies by a factor of ten among bones⁹⁷. Given its high
316 remodeling rate, the mandible would be expected to have high blood flow rates. The lone data
317 concerning mandible blood flow show values for the mandible that are similar to long bones^{97, 98}.
318 Importantly, however, these flow rates in the mandible are probably underestimated as the teeth
319 likely account for a significant portion of the mass, yet do not directly receive blood. Regardless
320 of basal blood flow, it would be expected that blood flow to the mandible would be reduced with
321 bisphosphonates due to the suppression of remodeling (which would lower the metabolic
322 demand). This reduced blood flow would lead to vascular remodeling⁹⁹ with the skeletal vessels
323 becoming smaller and thus less able to accommodate the demands for skeletal perfusion that are
324 known to exist post-extraction or with infection^{100, 101}. The inability to raise blood flow in these
325 circumstances could compromise tissue viability and play a role in BRONJ.

326

327 **BRONJ and Infection: It's there but does it contribute to the pathophysiology**

328 Numerous bacteria have been reported in patients with BRONJ yet there is nearly a universal
329 presence of *Actinomyces*^{68, 86, 87 102}. *Actinomyces* species, most commonly *Actinomyces israelii*,
330 are the most prominent of the over 500 microflora in the oral cavity¹⁰³. Through their formation of
331 a biofilm on the bone/tooth/mucosal surface, *Actinomyces* perpetuate the adherence of other

332 microflora which results in a heterogeneous population of bacteria primed for development of
333 infection ¹⁰³. Despite the presence of these bacterial conglomerates in many patients with
334 BRONJ, there is no clear evidence to address the question of whether infection is a primary or
335 secondary event in BRONJ pathophysiology.

336 One plausible mechanism through which infection could contribute to BRONJ is by
337 enhancing osteoclast-independent bone resorption. BRONJ tissue consistently shows a
338 prevalence of scalloped bone surfaces ^{68, 86, 87} (**Figure 5**), a seemingly paradoxical property given
339 the effect of bisphosphonates on bone resorption. Bacteria and associated fibroblast-like cells
340 have the capacity to directly resorb bone, independent of osteoclasts, by liberating various acids
341 and proteases ^{102, 104, 105}. As osteoclasts signal osteoblasts during normal bone remodeling ¹⁰⁶,
342 ¹⁰⁷, resorption that occurs independent of osteoclasts would likely lack osteoblast-mediated bone
343 formation. Whether such resorption could factor into BRONJ is unclear but seems worth
344 exploring.

345

346 **Other hypotheses of BRONJ**

347 In addition to the hypotheses outlined above, numerous others exist mostly related to the role of
348 various co-factors in the pathophysiology of BRONJ. Co-morbidities (e.g. diabetes ¹⁰⁸), lifestyle
349 factors (e.g. smoking ¹⁰⁹ and obesity ¹⁰⁹), interventions (e.g. dental extraction ¹⁰), and concurrent
350 medications (e.g. corticosteroids (¹¹⁰)) have all been associated with BRONJ. With all of these
351 factors, the proposed mechanism for contribution to BRONJ relates back to the main
352 mechanisms outlined above – remodeling, angiogenesis/blood flow, and infection. As dental
353 manifestations similar to BRONJ have not been observed with any of these co-factors in the
354 absence of bisphosphonates, it suggests either these co-factors don't play a significant role or
355 that it is the interaction between the co-factors and bisphosphonates that is the key to the
356 pathophysiology.

357

358 **Future Directions: Data anyone?**

359 Above all else, the field of BRONJ needs data. The amount of data, excluding those concerning
360 incidence/prevalence/risk factors, is appalling given the five years that have passed since the
361 initial descriptions of this condition. Without undertaking hypothesis-driven studies to tease apart
362 the potential pathophysiology we simply won't get any closer to understanding this condition.
363 Recently, the American Society for Bone and Mineral Research organized a multi-disciplinary
364 task force concerning BRONJ which put forward several questions/areas of study, ranging from
365 clinical to molecular, that the field needs to advance ¹. While this provides an excellent starting
366 point, the topics outlined are not all encompassing; other important areas related to BRONJ
367 surely exist. The key is that we need to start generating data, without which interest in BRONJ
368 within the research community will wane as the field will simply not move forward. We are
369 dangerously close to this happening and for the sake of the patients with BRONJ we must do
370 everything we can to understand all that we can.

371

372

373 **Figure Legends**

374

375 **Figure 1. The pathophysiology of BRONJ likely involves one or more of the bone cell**
376 **populations.** Osteoclasts (A), seen here stained with tartrate-resistant acid phosphatase,
377 function to resorb bone; suppression of their activity is the mechanism underlying the
378 effectiveness of bisphosphonate treatment. Osteocytes (B), entombed within the mineralized
379 matrix, are connected to each other and to the bone surface by an intricate cell process network
380 (seen here stained with basic fuchsin); the effect of bisphosphonates on these cells remains
381 controversial. Osteoblasts (C), seen here as tall cuboidal cells actively forming osteoid (the thin
382 pale blue seam adjacent to the bone surface), are less active in the presence of bisphosphonates
383 although this is predominately an indirect effect of reduced bone remodeling. Scale bars = 50 μ m.

384

385 **Figure 2. Bisphosphonates reduce mandible bone remodeling.** Following three years of
386 daily treatment with oral alendronate (ALN), at the dose used for osteoporosis treatment (ALN

387 0.2) or a dose 5x higher (ALN1.0), intracortical bone formation rate was assessed in the mandible
388 of female beagle dogs. There was a significant reduction in the overall bone formation rate of the
389 mandible with both doses of alendronate compared to age-matched animals treated with vehicle.
390 The greatest suppression of turnover was noted in the alveolar portion of the mandible with no
391 significant effect of ALN treatment on turnover suppression in the non-alveolar portion. * $p < 0.05$
392 versus VEH. *Adapted from J Oral Maxillofac Surg, 66(5), MR Allen and DB Burr, Mandible Matrix*
393 *Necrosis in Beagle Dogs After 3 Years of Daily Oral Bisphosphonate Treatment, 2008, with*
394 *permission from Elsevier.*

395

396 **Figure 3. The osteocyte lacunar-canalicular network.** Using acid etching of plastic embedded
397 specimens, the intricate nature of the lacunar-canalicular system can be revealed. Disruption of
398 this network could play a significant role in the pathophysiology of BRONJ. Scale bar = 50 μm .
399 Image complements of Daniel Kubek, Indiana University School of Medicine.

400

401 **Figure 4. Mandible matrix necrosis following bisphosphonate treatment in a pre-clinical**
402 **model.** Following three years of daily treatment with alendronate (ALN), regions of focal matrix
403 necrosis existed in the mandibles of beagle dogs. Using en bloc basic fuchsin staining, which
404 passively diffuses and fills all void spaces (blood vessels, lacunae, canaliculi), viable bone matrix
405 tissue can easily be identified by the presence of stain; the absence of stain indicates the lack of
406 permeability to a given region. In this representative photomicrograph of a mandible from an
407 ALN-treated animal, the central region is noticeably void of stain and therefore considered to be
408 non-viable tissue. Peripheral to the central region of non-viable bone matrix, tissue that is
409 sufficiently stained (and therefore considered viable) can be observed. The upper right of the
410 photomicrograph shows the tooth, below which is the periodontal ligament (which is heavily
411 stained with fuchsin). Scale bar = 500 μm .

412

413 **Figure 5. Non-viable bone matrix in BRONJ specimens.** Using en bloc basic fuchsin staining,
414 regions of non-viable bone matrix can be observed in a pathological specimen from a patient with
415 BRONJ (courtesy of Dr. Salvatore Ruggiero). Similar to that observed in bisphosphonate-treated
416 beagle dogs, a region void of fuchsin stain, and therefore considered non-viable, is surrounded by
417 stained (viable) tissue. Scale bar = 100 μ m.

418

419 **Figure 6. Extensive scalloped bone surfaces in BRONJ tissue.** Using high-resolution micro-
420 computed tomography (Skyscan 1172, 5 μ m resolution), the extent of eroded surfaces (examples
421 shown by arrows) in a sequestrum from a patient with BRONJ (courtesy of Dr. Salvatore
422 Ruggiero) can be visualized. Such extensive erosion would be unexpected in patients treated
423 with bisphosphonates, suggesting osteoclast-independent mechanisms of bone resorption may
424 be active in BRONJ. Scale bar = 1 mm.

425

426

427 **References**

- 428 1. Khosla, S., D. Burr, J. Cauley, et al. Bisphosphonate-associated osteonecrosis of the jaw:
429 report of a task force of the American Society for Bone and Mineral Research. *J Bone*
430 *Miner Res.* 22(10): 1479-91. 2007.
- 431 2. Marx, R.E. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis
432 of the jaws: a growing epidemic. *J Oral Maxillofac Surg.* 61(9): 1115-7. 2003.
- 433 3. Mehrotra, B., J. Fantasia, S. Nissel-Horowitz, et al. Osteonecrosis of the maxilla: an
434 unusual complication of prolonged bisphosphonate therapy. a case report. . *Proc Am Soc*
435 *Clin Oncol.* 22: (abstr 3194). 2003.
- 436 4. Ruggiero, S.L., B. Mehrotra, T.J. Rosenberg, et al. Osteonecrosis of the jaws associated
437 with the use of bisphosphonates: a review of 63 cases. *J Oral Maxillofac Surg.* 62(5):
438 527-34. 2004.
- 439 5. Wang, J., N.M. Goodger, and M.A. Pogrel. Osteonecrosis of the jaws associated with
440 cancer chemotherapy. *J Oral Maxillofac Surg.* 61(9): 1104-7. 2003.
- 441 6. Amler, M.H., P.L. Johnson, and I. Salman. Histological and histochemical investigation of
442 human alveolar socket healing in undisturbed extraction wounds. *J Am Dent Assoc.* 61:
443 32-44. 1960.
- 444 7. Cardaropoli, G., M. Araujo, and J. Lindhe. Dynamics of bone tissue formation in tooth
445 extraction sites. An experimental study in dogs. *J Clin Periodontol.* 30(9): 809-18. 2003.
- 446 8. Reid, I.R., M.J. Bolland, and A.B. Grey. Is bisphosphonate-associated osteonecrosis of
447 the jaw caused by soft tissue toxicity? *Bone.* 41(3): 318-20. 2007.
- 448 9. Reid, I.R. and T. Cundy. Osteonecrosis of the jaw. *Skeletal Radiol.* 2008.
- 449 10. Mavrokokki, T., A. Cheng, B. Stein, et al. Nature and frequency of bisphosphonate-
450 associated osteonecrosis of the jaws in Australia. *J Oral Maxillofac Surg.* 65(3): 415-23.
451 2007.
- 452 11. Rubegni, P. and M. Fimiani. Images in clinical medicine. Bisphosphonate-associated
453 contact stomatitis. *N Engl J Med.* 355(22): e25. 2006.

- 454 12. Reddy, G.T., T.M. Kumar, and Veena. Formulation and evaluation of Alendronate
455 Sodium gel for the treatment of bone resorptive lesions in Periodontitis. *Drug Deliv.* 12(4):
456 217-22. 2005.
- 457 13. Lin, J.H. Bisphosphonates: a review of their pharmacokinetic properties. *Bone.* 18(2): 75-
458 85. 1996.
- 459 14. Russell, R.G., N.B. Watts, F.H. Ebetino, et al. Mechanisms of action of bisphosphonates:
460 similarities and differences and their potential influence on clinical efficacy. *Osteoporos*
461 *Int.* 2008.
- 462 15. Russell, R.G., Z. Xia, J.E. Dunford, et al. Bisphosphonates: an update on mechanisms of
463 action and how these relate to clinical efficacy. *Ann N Y Acad Sci.* 1117: 209-57. 2007.
- 464 16. Rodan, G.A. and H.A. Fleisch. Bisphosphonates: mechanisms of action. *J Clin Invest.*
465 97(12): 2692-6. 1996.
- 466 17. Allen, M.R., K. Iwata, R. Phipps, et al. Alterations in canine vertebral bone turnover,
467 microdamage accumulation, and biomechanical properties following 1-year treatment
468 with clinical treatment doses of risedronate or alendronate. *Bone.* 39(4): 872-9. 2006.
- 469 18. Mashiba, T., C.H. Turner, T. Hirano, et al. Effects of suppressed bone turnover by
470 bisphosphonates on microdamage accumulation and biomechanical properties in
471 clinically relevant skeletal sites in beagles. *Bone.* 28(5): 524-31. 2001.
- 472 19. Allen, M.R. and D.B. Burr. Three years of alendronate treatment results in similar levels
473 of vertebral microdamage as after one year of treatment. *J Bone Miner Res.* 22(11):
474 1759-65. 2007.
- 475 20. Allen, M.R. and D.B. Burr. Mandible matrix necrosis in beagle dogs after 3 years of daily
476 oral bisphosphonate treatment. *J Oral Maxillofac Surg.* 66(5): 987-94. 2008.
- 477 21. Boivin, G.Y., P.M. Chavassieux, A.C. Santora, et al. Alendronate increases bone strength
478 by increasing the mean degree of mineralization of bone tissue in osteoporotic women.
479 *Bone.* 27(5): 687-94. 2000.
- 480 22. McDonald, M.M., S. Dulai, C. Godfrey, et al. Bolus or weekly zoledronic acid
481 administration does not delay endochondral fracture repair but weekly dosing enhances
482 delays in hard callus remodeling. *Bone.* 2008.
- 483 23. Plotkin, L.I., J.I. Aguirre, S. Kousteni, et al. Bisphosphonates and estrogens inhibit
484 osteocyte apoptosis via distinct molecular mechanisms downstream of extracellular
485 signal-regulated kinase activation. *J Biol Chem.* 280(8): 7317-25. 2005.
- 486 24. Idris, A.I., J. Rojas, I.R. Greig, et al. Aminobisphosphonates cause osteoblast apoptosis
487 and inhibit bone nodule formation in vitro. *Calcif Tissue Int.* 82(3): 191-201. 2008.
- 488 25. Follet, H., J. Li, R.J. Phipps, et al. Risedronate and alendronate suppress osteocyte
489 apoptosis following cyclic fatigue loading. *Bone.* 40(4): 1172-7. 2007.
- 490 26. Plotkin, L.I., R.S. Weinstein, A.M. Parfitt, et al. Prevention of osteocyte and osteoblast
491 apoptosis by bisphosphonates and calcitonin. *J Clin Invest.* 104(10): 1363-74. 1999.
- 492 27. Boyce, R.W., C.L. Paddock, J.R. Gleason, et al. The effects of risedronate on canine
493 cancellous bone remodeling: three-dimensional kinetic reconstruction of the remodeling
494 site. *J Bone Miner Res.* 10(2): 211-21. 1995.
- 495 28. Eriksen, E.F., F. Melsen, E. Sod, et al. Effects of long-term risedronate on bone quality
496 and bone turnover in women with postmenopausal osteoporosis. *Bone.* 31(5): 620-5.
497 2002.
- 498 29. Iwata, K., J. Li, H. Follet, et al. Bisphosphonates suppress periosteal osteoblast activity
499 independent of resorption in rat femur and tibia. *Bone.* 39(5): 1053-8. 2006.
- 500 30. Allen, M.R., H. Follet, M. Khurana, et al. Anti-remodeling agents influence osteoblast
501 activity differently in modeling- and remodeling-associated bone formation *Calcified*
502 *Tissue International.* 79(4): 255-61. 2006.
- 503 31. Allen, M.R., S. Reinwald, and D.B. Burr. Alendronate reduces bone toughness of ribs
504 without significantly increasing microdamage accumulation in dogs following 3 years of
505 daily treatment. *Calcif Tissue Int.* 82(5): 354-60. 2008.
- 506 32. Mashiba, T., T. Hirano, C.H. Turner, et al. Suppressed bone turnover by
507 bisphosphonates increases microdamage accumulation and reduces some
508 biomechanical properties in dog rib. *J Bone Miner Res.* 15(4): 613-20. 2000.

- 509 33. Reinwald, S. and D. Burr. Review of nonprimate, large animal models for osteoporosis
510 research. *J Bone Miner Res.* 23(9): 1353-68. 2008.
- 511 34. Garetto, L.P., J. Chen, J.A. Parr, et al. Remodeling dynamics of bone supporting rigidly
512 fixed titanium implants: a histomorphometric comparison in four species including
513 humans. *Implant Dent.* 4(4): 235-43. 1995.
- 514 35. Han, Z.H., S. Palnitkar, D.S. Rao, et al. Effects of ethnicity and age or menopause on the
515 remodeling and turnover of iliac bone: implications for mechanisms of bone loss. *J Bone
516 Miner Res.* 12(4): 498-508. 1997.
- 517 36. Huja, S.S., S.A. Fernandez, K.J. Hill, et al. Remodeling dynamics in the alveolar process
518 in skeletally mature dogs. *Anat Rec A Discov Mol Cell Evol Biol.* 288(12): 1243-9. 2006.
- 519 37. Garetto, L.P. and N.D. Tricker. 1998 Remodeling of bone surrounding the implant
520 interface., in *Bridging the Gap Between Dental & Orthopaedic Implants*, 3rd Annual
521 Indiana Conference, L.P. Garetto, et al., Editors: Indianapolis, IN.
- 522 38. Bamias, A., E. Kastritis, C. Bamia, et al. Osteonecrosis of the jaw in cancer after
523 treatment with bisphosphonates: incidence and risk factors. *J Clin Oncol.* 23(34): 8580-7.
524 2005.
- 525 39. Dimopoulos, M.A., E. Kastritis, A. Anagnostopoulos, et al. Osteonecrosis of the jaw in
526 patients with multiple myeloma treated with bisphosphonates: evidence of increased risk
527 after treatment with zoledronic acid. *Haematologica.* 91(7): 968-71. 2006.
- 528 40. Corso, A., M. Varettoni, P. Zappasodi, et al. A different schedule of zoledronic acid can
529 reduce the risk of the osteonecrosis of the jaw in patients with multiple myeloma.
530 *Leukemia.* 2007.
- 531 41. Lewiecki, E.M., P.D. Miller, M.R. McClung, et al. Two-year treatment with denosumab
532 (AMG 162) in a randomized phase 2 study of postmenopausal women with low BMD. *J
533 Bone Miner Res.* 22(12): 1832-41. 2007.
- 534 42. Lipton, A., G.G. Steger, J. Figueroa, et al. Randomized active-controlled phase II study of
535 denosumab efficacy and safety in patients with breast cancer-related bone metastases. *J
536 Clin Oncol.* 25(28): 4431-7. 2007.
- 537 43. Helfrich, M.H. Osteoclast diseases and dental abnormalities. *Arch Oral Biol.* 50(2): 115-
538 22. 2005.
- 539 44. Barry, C.P., C.D. Ryan, and L.F. Stassen. Osteomyelitis of the maxilla secondary to
540 osteopetrosis: a report of 2 cases in sisters. *J Oral Maxillofac Surg.* 65(1): 144-7. 2007.
- 541 45. Johnston, C.C., Jr., N. Lavy, T. Lord, et al. Osteopetrosis. A clinical, genetic, metabolic,
542 and morphologic study of the dominantly inherited, benign form. *Medicine (Baltimore).*
543 47(2): 149-67. 1968.
- 544 46. Waguespack, S.G., D.L. Koller, K.E. White, et al. Chloride channel 7 (CLCN7) gene
545 mutations and autosomal dominant osteopetrosis, type II. *J Bone Miner Res.* 18(8): 1513-
546 8. 2003.
- 547 47. Ruggiero, S.L. and S.J. Drew. Osteonecrosis of the jaws and bisphosphonate therapy. *J
548 Dent Res.* 86(11): 1013-21. 2007.
- 549 48. Bathi, R.J. and V.N. Masur. Pyknodysostosis--a report of two cases with a brief review of
550 the literature. *Int J Oral Maxillofac Surg.* 29(6): 439-42. 2000.
- 551 49. Dimitrakopoulos, I., C. Magopoulos, and T. Katopodi. Mandibular osteomyelitis in a
552 patient with pyknodysostosis: a case report of a 50-year misdiagnosis. *J Oral Maxillofac
553 Surg.* 65(3): 580-5. 2007.
- 554 50. Harper, R.P. and E. Fung. Resolution of bisphosphonate-associated osteonecrosis of the
555 mandible: possible application for intermittent low-dose parathyroid hormone [rhPTH(1-
556 34)]. *J Oral Maxillofac Surg.* 65(3): 573-80. 2007.
- 557 51. Lau, A.N., S.H. Ali, and J.D. Adachi. Resolution of osteonecrosis of the jaw after
558 teriparatide [recombinant human PTH (1-34)] therapy. *Osteoporos Int.* 19 (Suppl 1): S80.
559 2008.
- 560 52. Wang, H.L., D. Weber, and L.K. McCauley. Effect of long-term oral bisphosphonates on
561 implant wound healing: literature review and a case report. *J Periodontol.* 78(3): 584-94.
562 2007.

- 563 53. Malmgren, B., E. Astrom, and S. Soderhall. No osteonecrosis in jaws of young patients
564 with osteogenesis imperfecta treated with bisphosphonates. *J Oral Pathol Med.* 37(4):
565 196-200. 2008.
- 566 54. Schwartz, S., C. Joseph, D. Iera, et al. Bisphosphonates, osteonecrosis, osteogenesis
567 imperfecta and dental extractions: a case series. *J Can Dent Assoc.* 74(6): 537-42. 2008.
- 568 55. Kingsmill, V.J. Post-extraction remodeling of the adult mandible. *Crit Rev Oral Biol Med.*
569 10(3): 384-404. 1999.
- 570 56. Li, C., S. Mori, J. Li, et al. Long-term effect of incadronate disodium (YM-175) on fracture
571 healing of femoral shaft in growing rats. *J Bone Miner Res.* 16(3): 429-36. 2001.
- 572 57. Li, J., S. Mori, Y. Kaji, et al. Concentration of bisphosphonate (incadronate) in callus area
573 and its effects on fracture healing in rats. *J Bone Miner Res.* 15(10): 2042-51. 2000.
- 574 58. Peter, C.P., W.O. Cook, D.M. Nunamaker, et al. Effect of alendronate on fracture healing
575 and bone remodeling in dogs. *J Orthop Res.* 14(1): 74-9. 1996.
- 576 59. Altundal, H. and O. Guvener. The effect of alendronate on resorption of the alveolar bone
577 following tooth extraction. *Int J Oral Maxillofac Surg.* 33(3): 286-93. 2004.
- 578 60. Olson, H.M. and A. Hagen. Inhibition of post-extraction alveolar ridge resorption in rats by
579 dichloromethane diphosphonate. *J Periodontal Res.* 17(6): 669-74. 1982.
- 580 61. Bonewald, L.F. Osteocytes as dynamic multifunctional cells. *Ann N Y Acad Sci.* 1116:
581 281-90. 2007.
- 582 62. Turner, C.H., A.G. Robling, R.L. Duncan, et al. Do bone cells behave like a neuronal
583 network? *Calcif Tissue Int.* 70(6): 435-42. 2002.
- 584 63. Weinstein, R.S., R.L. Jilka, A.M. Parfitt, et al. Inhibition of osteoblastogenesis and
585 promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential
586 mechanisms of their deleterious effects on bone. *J Clin Invest.* 102(2): 274-82. 1998.
- 587 64. Enlow, D.H. Functions of the Haversian system. *Am J Anat.* 110: 269-305. 1962.
- 588 65. Frost, H.M. In vivo osteocyte death. *J Bone Joint Surg Am.* 42-A: 138-43. 1960.
- 589 66. Burr, D.B. and M. Hooser. Alterations to the en bloc basic fuchsin staining protocol for the
590 demonstration of microdamage produced in vivo. *Bone.* 17(4): 431-3. 1995.
- 591 67. Frost, H.M. Micropetrosis. *J Bone Joint Surg Am.* 42-A: 144-50. 1960.
- 592 68. Hansen, T., M. Kunkel, A. Weber, et al. Osteonecrosis of the jaws in patients treated with
593 bisphosphonates - histomorphologic analysis in comparison with infected
594 osteoradionecrosis. *J Oral Pathol Med.* 35(3): 155-60. 2006.
- 595 69. Allen, M.R. Bisphosphonates and Osteonecrosis of the Jaw: Moving From the 'Bedside'
596 to the 'Bench'. *Cells, Tissues, Organs.* In Press. 2008.
- 597 70. Masarachia, P., M. Weinreb, R. Balena, et al. Comparison of the distribution of 3H-
598 alendronate and 3H-etidronate in rat and mouse bones. *Bone.* 19(3): 281-90. 1996.
- 599 71. Lin, J.H., D.E. Duggan, I.W. Chen, et al. Physiological disposition of alendronate, a
600 potent anti-osteolytic bisphosphonate, in laboratory animals. *Drug Metab Dispos.* 19(5):
601 926-32. 1991.
- 602 72. Roelofs, A.J., F.P. Coxon, F.H. Ebetino, et al. Use of a fluorescent analogue of
603 risedronate to study localization and cellular uptake of bisphosphonates in vivo. *Bone.* 42:
604 S85. 2008.
- 605 73. Plotkin, L.I., S.C. Manolagas, and T. Bellido. Transduction of cell survival signals by
606 connexin-43 hemichannels. *J Biol Chem.* 277(10): 8648-57. 2002.
- 607 74. Plotkin, L.I., S.C. Manolagas, and T. Bellido. Dissociation of the pro-apoptotic effects of
608 bisphosphonates on osteoclasts from their anti-apoptotic effects on
609 osteoblasts/osteocytes with novel analogs. *Bone.* 39(3): 443-52. 2006.
- 610 75. Plotkin, L.I., V. Lezcano, J. Thostenson, et al. Connexin 43 Is Required for the Anti-
611 Apoptotic Effect of Bisphosphonates on Osteocytes and Osteoblasts In Vivo. *J Bone*
612 *Miner Res.* 2008.
- 613 76. Nancollas, G.H., R. Tang, R.J. Phipps, et al. Novel insights into actions of
614 bisphosphonates on bone: Differences in interactions with hydroxyapatite. *Bone.* 2005.
- 615 77. Leu, C.T., E. Luegmayer, L.P. Freedman, et al. Relative binding affinities of
616 bisphosphonates for human bone and relationship to antiresorptive efficacy. *Bone.* 38(5):
617 628-36. 2006.

- 618 78. Kim, H.K. Introduction to osteonecrosis of the femoral head (OFH) and osteonecrosis of
619 the jaw (ONJ). *J Musculoskelet Neuronal Interact.* 7(4): 350-3. 2007.
- 620 79. Store, G. and M. Boysen. Mandibular osteoradionecrosis: clinical behaviour and
621 diagnostic aspects. *Clin. Otolaryngol.* 25: 378-384. 2000.
- 622 80. Store, G. and G. Grandstrom. Osteoradionecrosis of the mandible: a microradiographic
623 study of cortical bone. *Scan J Plast Reconstr Hand Surg.* 33: 307-314. 1999.
- 624 81. Guise, T.A. Antitumor effects of bisphosphonates: promising preclinical evidence. *Cancer*
625 *Treat Rev.* 34 Suppl 1: S19-24. 2008.
- 626 82. Lipton, A. Emerging role of bisphosphonates in the clinic--antitumor activity and
627 prevention of metastasis to bone. *Cancer Treat Rev.* 34 Suppl 1: S25-30. 2008.
- 628 83. Wood, J., K. Bonjean, S. Ruetz, et al. Novel antiangiogenic effects of the bisphosphonate
629 compound zoledronic acid. *J Pharmacol Exp Ther.* 302(3): 1055-61. 2002.
- 630 84. Fournier, P., S. Boissier, S. Filleur, et al. Bisphosphonates inhibit angiogenesis in vitro
631 and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats.
632 *Cancer Res.* 62(22): 6538-44. 2002.
- 633 85. Schenk, R., W.A. Merz, R. Muhlbauer, et al. Effect of ethane-1-hydroxy-1,1-
634 diphosphonate (EHDP) and dichloromethylene diphosphonate (Cl 2 MDP) on the
635 calcification and resorption of cartilage and bone in the tibial epiphysis and metaphysis of
636 rats. *Calcif Tissue Res.* 11(3): 196-214. 1973.
- 637 86. Hellstein, J.W. and C.L. Marek. Bisphosphonate osteochemonecrosis (bis-phossy jaw): is
638 this phossy jaw of the 21st century? *J Oral Maxillofac Surg.* 63(5): 682-9. 2005.
- 639 87. Hansen, T., M. Kunkel, E. Springer, et al. Actinomycosis of the jaws--histopathological
640 study of 45 patients shows significant involvement in bisphosphonate-associated
641 osteonecrosis and infected osteoradionecrosis. *Virchows Arch.* 451(6): 1009-17. 2007.
- 642 88. Estilo, C.L., M. Fournier, A. Farooki, et al. Osteonecrosis of the jaw related to
643 bevacizumab. *J Clin Oncol.* 26(24): 4037-8. 2008.
- 644 89. Parfitt, A.M. The mechanism of coupling: a role for the vasculature. *Bone.* 26(4): 319-23.
645 2000.
- 646 90. Somerman, M.J. and L.K. McCauley. Bisphosphonates: Sacrificing the jaw to save the
647 skeleton? *BoneKEy-Osteovision.* 3(9): 12-18. 2006.
- 648 91. Adeyemo, W.L., A.L. Ladeinde, and M.O. Ogunlewe. Clinical evaluation of post-extraction
649 site wound healing. *J Contemp Dent Pract.* 7(3): 40-9. 2006.
- 650 92. Amler, M.H. Disturbed healing of extraction wounds. *J Oral Implantol.* 25(3): 179-84.
651 1999.
- 652 93. Trombelli, L., R. Farina, A. Marzola, et al. Modeling and remodeling of human extraction
653 sockets. *J Clin Periodontol.* 35(7): 630-9. 2008.
- 654 94. Deckers, M.M., E.R. Van Beek, G. Van Der Pluijm, et al. Dissociation of angiogenesis
655 and osteoclastogenesis during endochondral bone formation in neonatal mice. *J Bone*
656 *Miner Res.* 17(6): 998-1007. 2002.
- 657 95. Tothill, P. and J.N. MacPherson. The distribution of blood flow to the whole skeleton in
658 dogs, rabbits and rats measured with microspheres. *Clin Phys Physiol Meas.* 7(2): 117-
659 23. 1986.
- 660 96. Rowell, L.B. 1993 *Human Cardiovascular Control.* New York: Oxford University Press.
- 661 97. Colleran, P.N., M.K. Wilkerson, S.A. Bloomfield, et al. Alterations in skeletal perfusion
662 with simulated microgravity: a possible mechanism for bone remodeling. *J Appl Physiol.*
663 89(3): 1046-54. 2000.
- 664 98. Bloomfield, S.A., H.A. Hogan, and M.D. Delp. Decreases in bone blood flow and bone
665 material properties in aging Fischer-344 rats. *Clin Orthop Relat Res.* (396): 248-57. 2002.
- 666 99. Delp, M.D., P.N. Colleran, M.K. Wilkerson, et al. Structural and functional remodeling of
667 skeletal muscle microvasculature is induced by simulated microgravity. *Am J Physiol*
668 *Heart Circ Physiol.* 278(6): H1866-73. 2000.
- 669 100. Lurie, A.G. and S.R. Matteson. 99mTc-diphosphonate bone imaging and uptake in
670 healing rat extraction sockets. *J Nucl Med.* 17(8): 688-92. 1976.
- 671 101. McDougall, I.R. Skeletal scintigraphy. *West J Med.* 130(6): 503-14. 1979.

- 672 102. Sedghizadeh, P.P., S.K. Kumar, A. Gorur, et al. Identification of microbial biofilms in
673 osteonecrosis of the jaws secondary to bisphosphonate therapy. *J Oral Maxillofac Surg.*
674 66(4): 767-75. 2008.
- 675 103. Yeung, M.K. Molecular and genetic analyses of *Actinomyces* spp. *Crit Rev Oral Biol Med.*
676 10(2): 120-38. 1999.
- 677 104. Nair, S.P., S. Meghji, M. Wilson, et al. Bacterially induced bone destruction: mechanisms
678 and misconceptions. *Infect Immun.* 64(7): 2371-80. 1996.
- 679 105. Pap, T., A. Claus, S. Ohtsu, et al. Osteoclast-independent bone resorption by fibroblast-
680 like cells. *Arthritis Res Ther.* 5(3): R163-73. 2003.
- 681 106. Mundy, G.R. and F. Elefteriou. Boning up on ephrin signaling. *Cell.* 126(3): 441-3. 2006.
- 682 107. Zhao, C., N. Irie, Y. Takada, et al. Bidirectional ephrinB2-EphB4 signaling controls bone
683 homeostasis. *Cell Metab.* 4(2): 111-21. 2006.
- 684 108. Khamaisi, M., E. Regev, N. Yarom, et al. Possible association between diabetes and
685 bisphosphonate-related jaw osteonecrosis. *J Clin Endocrinol Metab.* 92(3): 1172-5. 2007.
- 686 109. Wessel, J.H., T.B. Dodson, and A.I. Zavras. Zoledronate, smoking, and obesity are
687 strong risk factors for osteonecrosis of the jaw: a case-control study. *J Oral Maxillofac*
688 *Surg.* 66(4): 625-31. 2008.
- 689 110. American Association of Oral and Maxillofacial Surgeons position paper on
690 bisphosphonate-related osteonecrosis of the jaws. *J Oral Maxillofac Surg.* 65(3): 369-76.
691 2007.
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693

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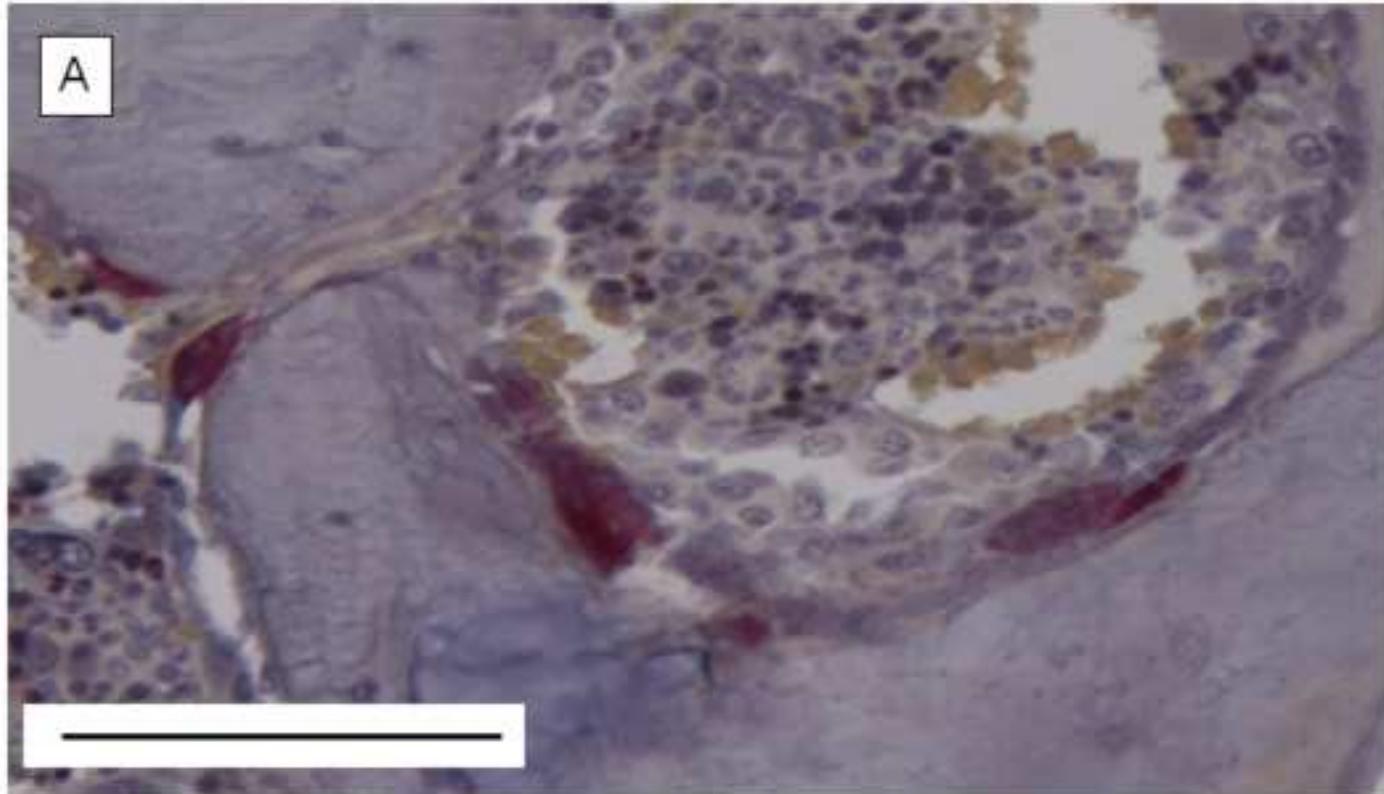


Figure 1a

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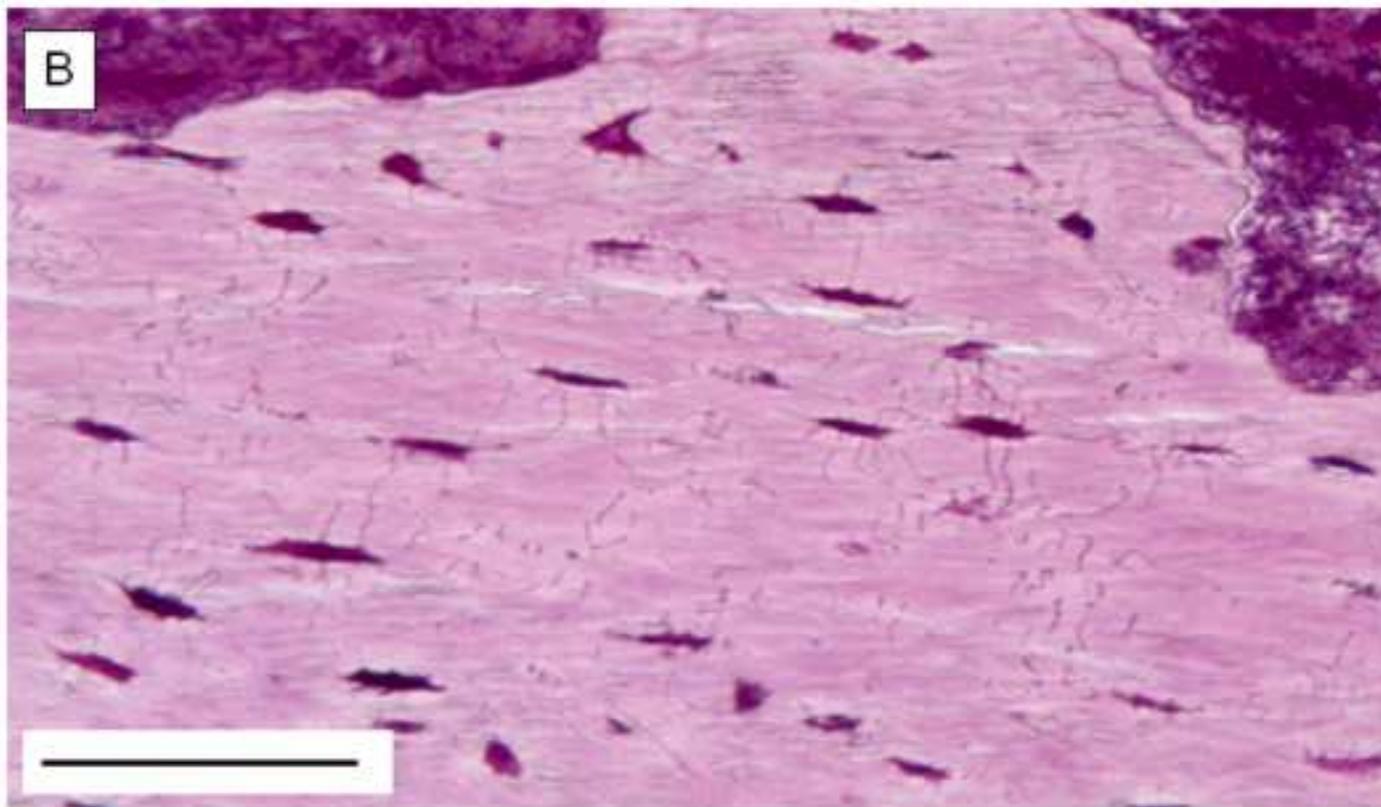


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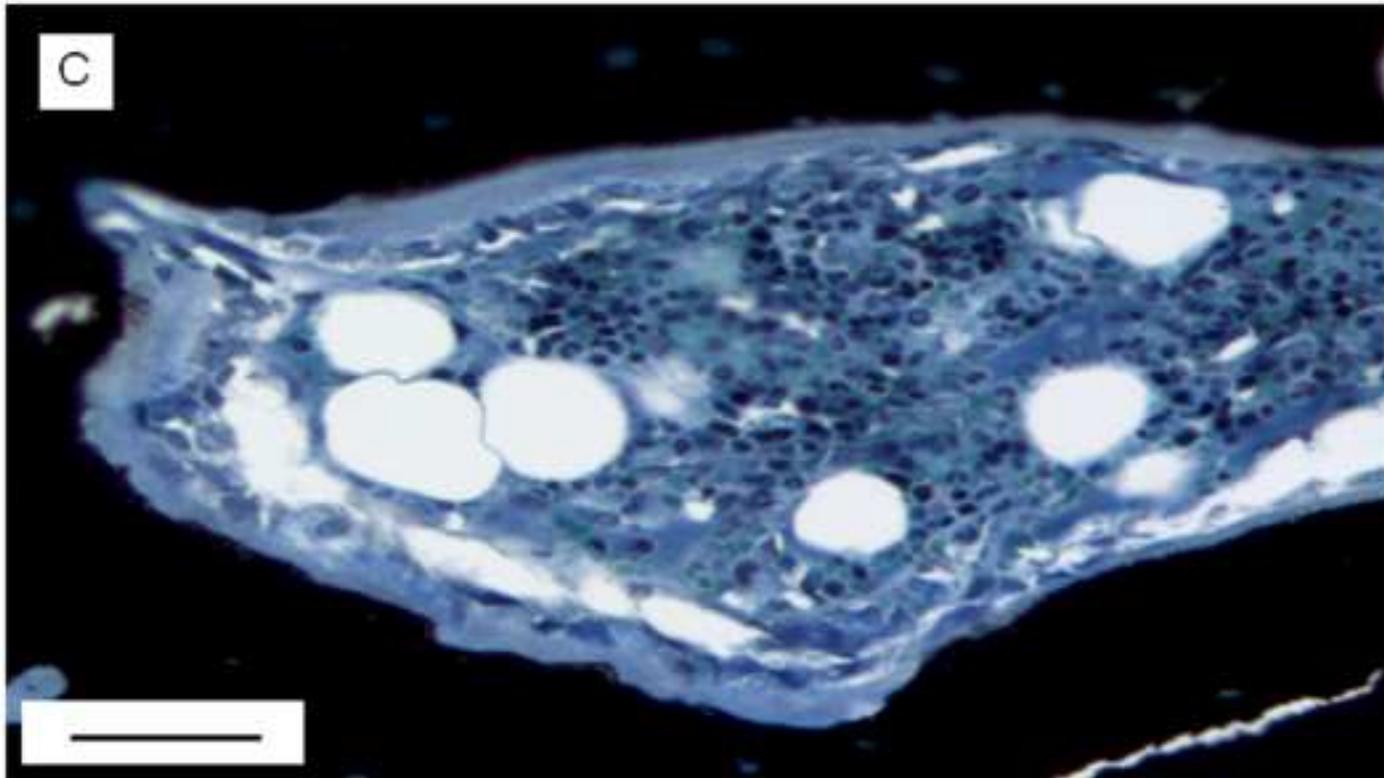


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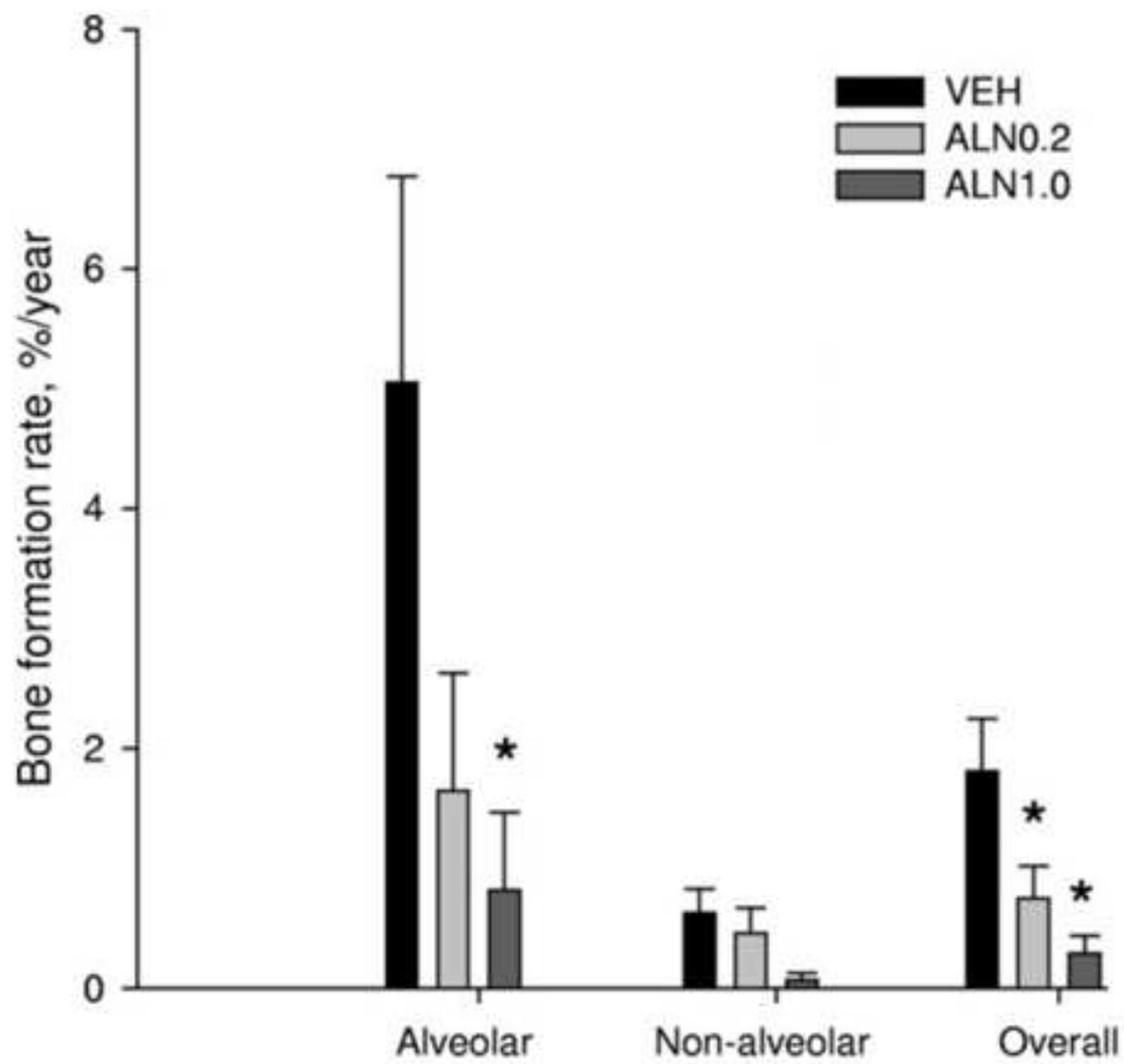


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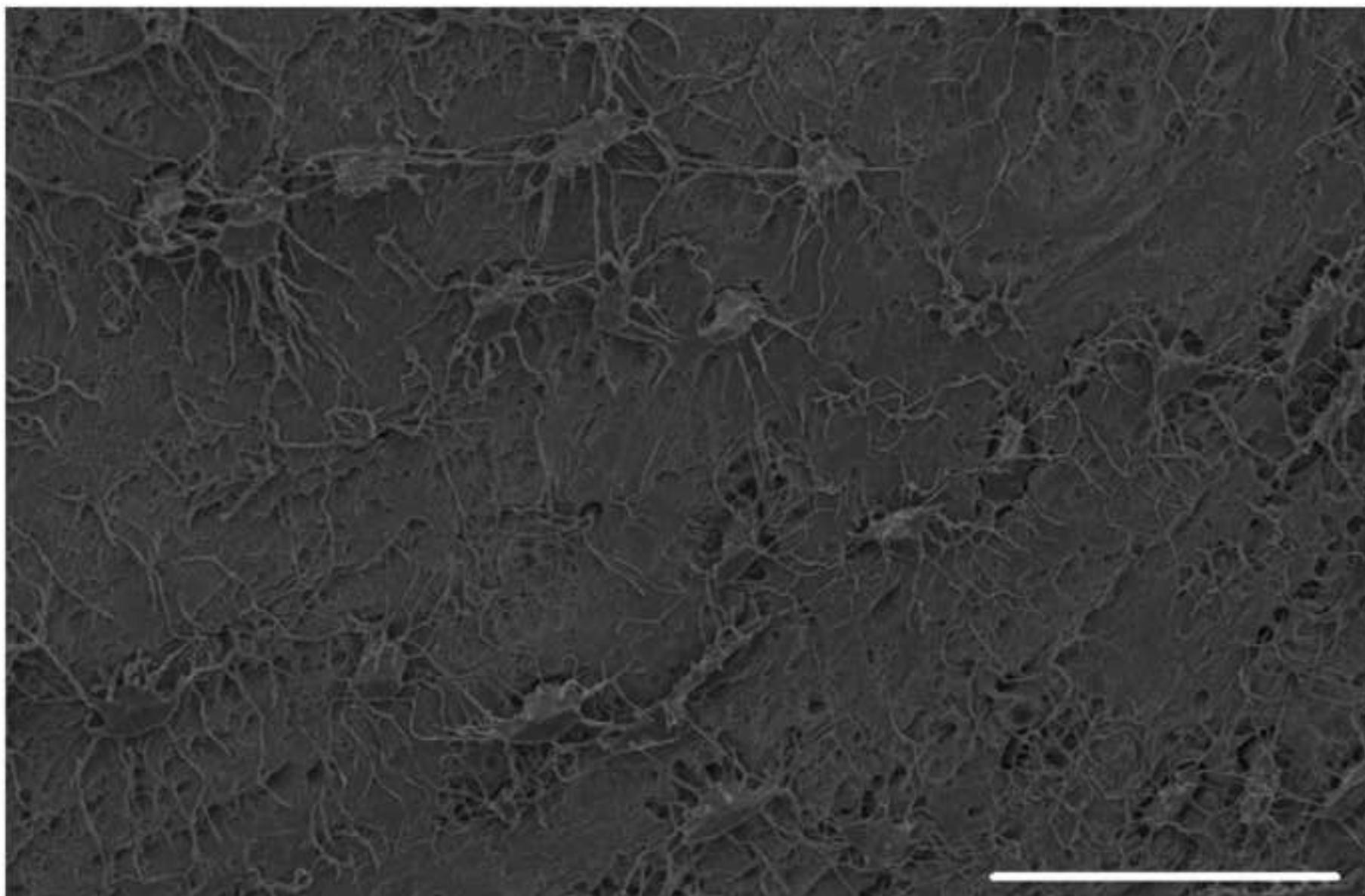


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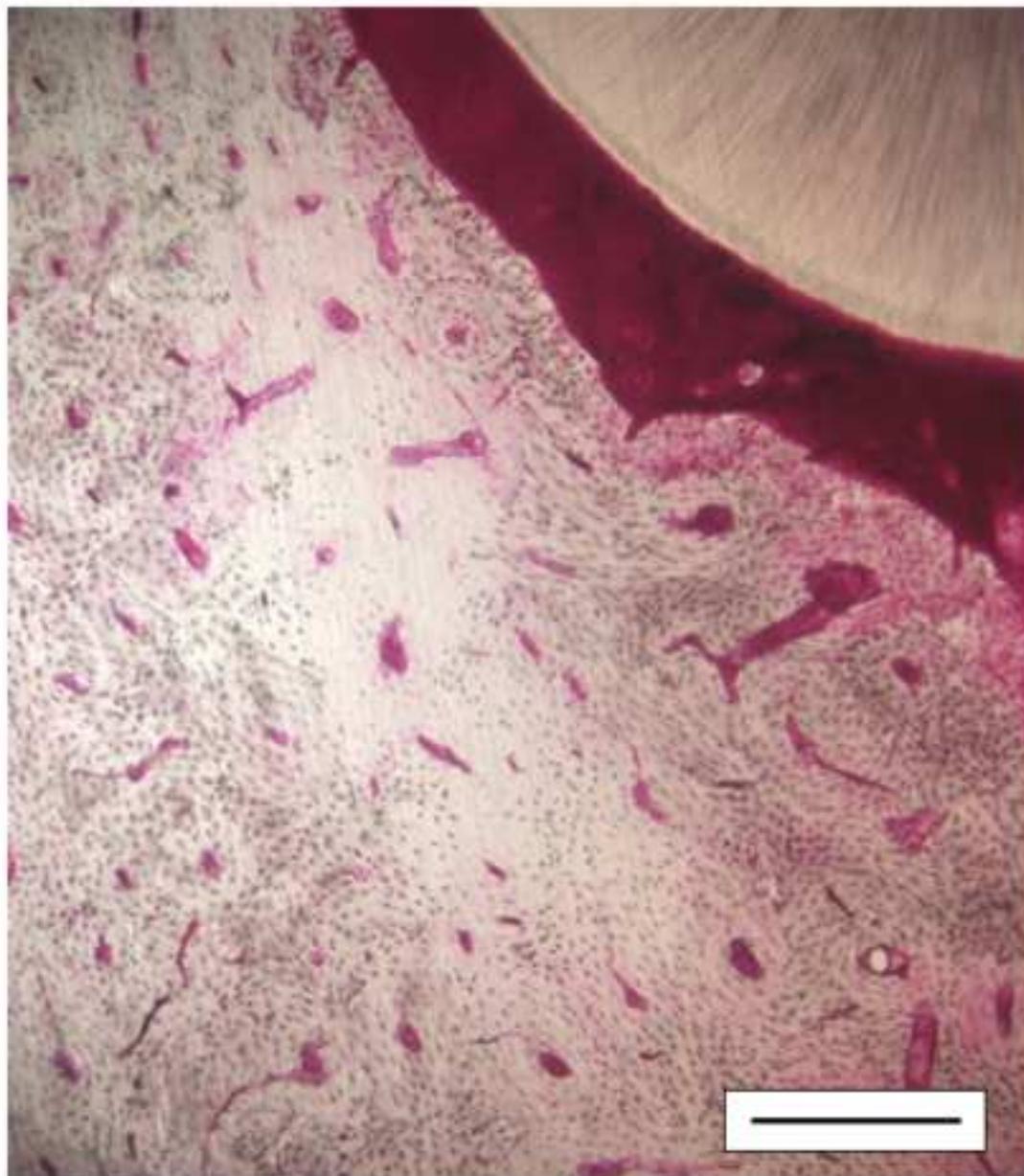


Figure 4

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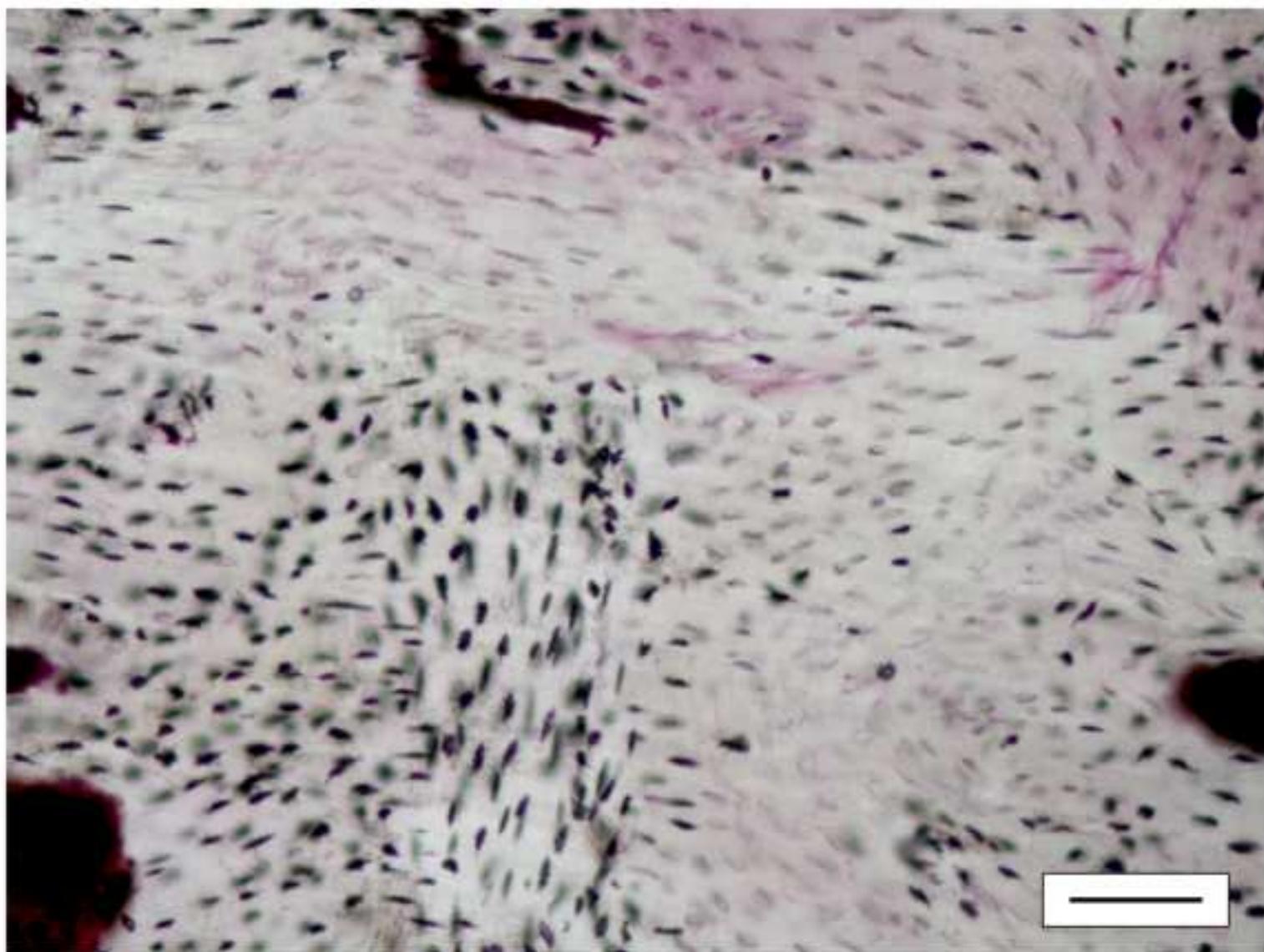


Figure 5

Figure 6
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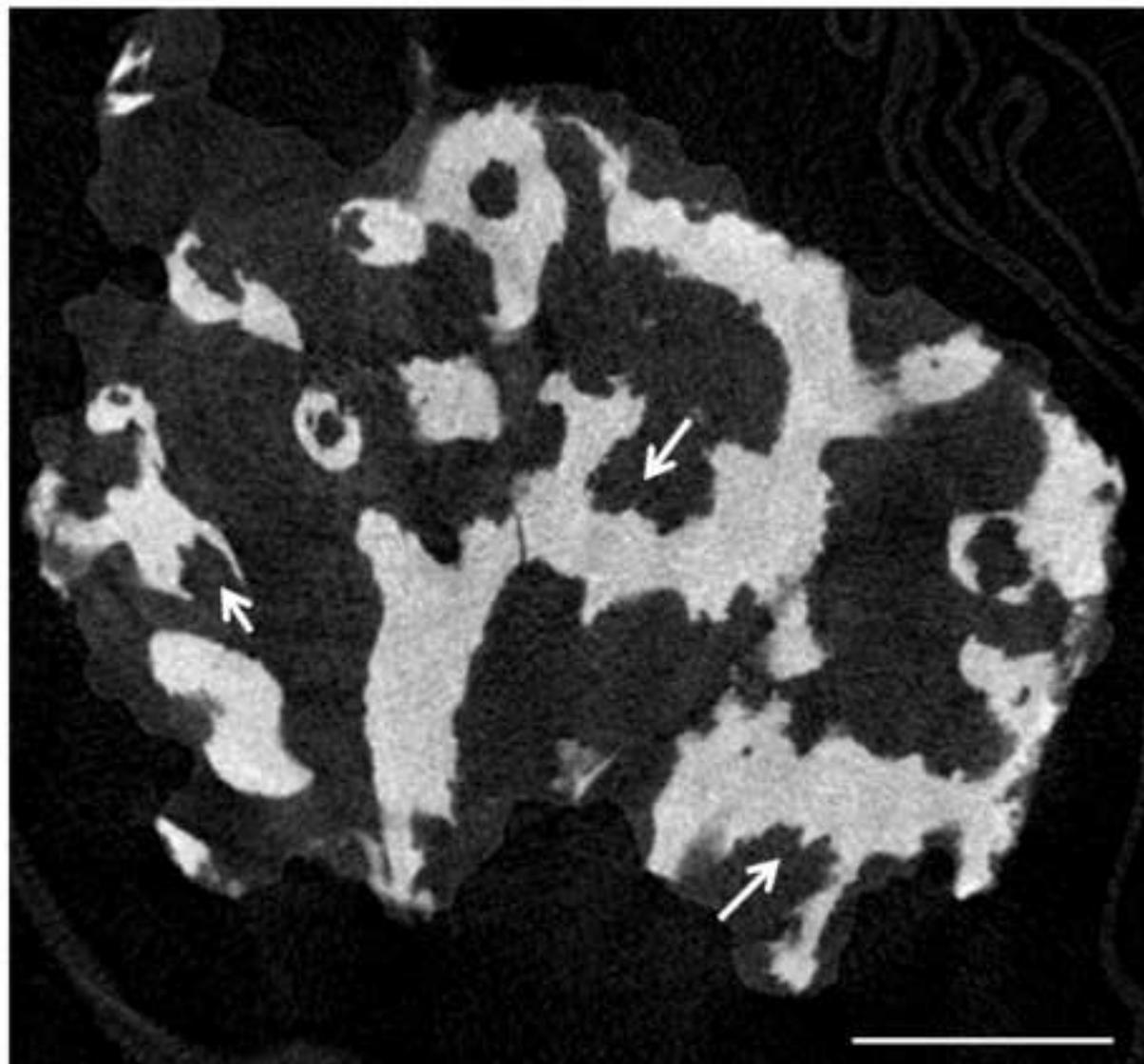


Figure 6