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

Matthew R. Allen [1] and David B. Burr [1,2,3]

[1] Department of Anatomy and Cell Biology

[2] Department of Orthopaedic Surgery, Indiana University School of Medicine

[3] Biomedical Engineering Program

Indiana University-Purdue University, Indianapolis

Indianapolis, IN 46202

Corresponding Author:

Matthew R. Allen

Dept of Anatomy & Cell Biology

635 Barnhill Drive, MS-5035

Indianapolis, IN 46202

Tel: (317) 274-1283

FAX: (317) 278-2040

Email: matallen@iupui.edu

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Abstract

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

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

Introduction

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

The starting point for BRONJ: Bone or soft tissue?

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

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

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

Bone cells and BRONJ: Where to focus attention

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

animal models suggest that bisphosphonates may suppress osteoblastic bone formation directly on those surface undergoing bone formation without prior resorption (i.e .formation modeling) [29], although large animal models do not show a similar suppressive effect on periosteal surfaces [30-32]. Collectively, this evidence points towards the osteoclasts and/or osteocytes as the main cells of interest for BRONJ pathogenesis.

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

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

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

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

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

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

An important aspect of the remodeling suppression hypothesis is that much of the focus has been on the pre-existing bone which may not be the true site of interest. Similar to fracture healing, after dental extraction the socket fills with woven bone which over time is remodeled into lamellar bone [6, 7, 55]. The fracture healing literature clearly shows woven bone formation is not compromised in the presence of bisphosphonates, yet remodeling of this callus is significantly delayed [22, 56-58]. Thus, it may be that bone formed early during oral wound healing is not remodeled in a timely fashion and this in turn develops into BRONJ. Although some investigation has occurred looking at extractions in the presence of bisphosphonates [59, 60], these studies have focused on the preservation of the alveolar bone structure. There exist no data to describe how 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 death [63-65]. Under normal physiological conditions, loss of osteocytes and
205 the associated changes to tissue can likely be held in check by bone remodeling [64]. 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 matrix [20]. 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 animals [20]. 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 bone [68]. 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

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

Whether these direct or indirect pathways connecting bisphosphonates to loss of osteocyte viability play a role in BRONJ is unclear. Of the two, the direct pathway is more consistent with numerous clinical aspects of the condition. BRONJ is more prominent in patients treated with high-doses of intravenous pamidronate or zoledronate, as compared to those treated at lower doses for osteoporosis [1]. Intravenous administration results in a higher skeletal accumulation as compared to oral administration [13], while pamidronate and zoledronate have the highest mineral binding affinities among all of the bisphosphonates [76, 77]. The increased risk of BRONJ associated with treatment duration is also consistent with the accumulation of drug over time.

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

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

Prior to the emergence of BRONJ, much of what was known concerning osteonecrosis centered on two conditions which manifest due to disruptions in vasculature. Avascular necrosis of the hip occurs secondary to disruption of the vasculature [78]. Similarly, osteoradionecrosis, most prominently of the jaw, occurs following radiation-induced disruption of the vasculature [68, 79, 80]. The existence of these conditions, and the clear role of disrupted vasculature in their pathophysiology, has led to the hypothesis that the vasculature plays a key role in pathophysiology of BRONJ.

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

documented anti-angiogenic effects of bisphosphonates in vitro while a smaller number have shown similar effects in vivo. The latter include suppression of angiogenesis in subcutaneously implanted tissue chambers [83], reduced testosterone-induced prostate tissue re-vascularization following castration [84], and significant reductions in marrow vessel number of iliac crest biopsies after six months of clodronate treatment for Paget's disease [84]. Conversely, early in vivo studies with high doses of bisphosphonates did not document altered vascular invasion near the growth plates [85]. There have been no systematic studies assessing the vascular pattern in BRONJ. Qualitatively, the vasculature has been reported to be intact in a series of BRONJ cases [86] while a separate series reported 'vessel obliteration' in some BRONJ specimens [68, 87]. In the dog model of matrix necrosis the vasculature appears to be patent and intact even in regions devoid of viable osteocytes [20].

Recently, two cases of exposed bone in the mandible, similar in nature to BRONJ, have been reported in cancer patients treated with Bevacizumab, a recombinant human monoclonal antibody that binds to vascular endothelial growth factor (VEGF) and inhibits angiogenesis [88]. These patients were not treated with bisphosphonates and did not undergo any dental intervention. This provides the strongest evidence to date suggesting a role of the vasculature in BRONJ.

Reduced angiogenesis within bone would actually be expected to occur with bisphosphonates, due to a suppression of remodeling. Each remodeling unit receives its nutrients by a vessel [89]; therefore bone remodeling and angiogenesis are intimately linked. Bisphosphonate-induced reductions in remodeling should be associated with reduced angiogenesis yet the reduction would be a secondary effect. It remains unclear if this has relevance to BRONJ.

Perhaps the most intriguing role of altered angiogenesis with bisphosphonates may be related to wound healing [90]. Following tooth extraction, a major precipitating event in BRONJ [10], the extraction site undergoes a well-defined series of healing steps which include an initial clot formation, conversion of clot to granulation tissue, formation of connective tissue and pre-osseous tissue, and finally filling of the extraction socket with bone [7, 91-93]. Disruption of this

normal process at any stage, particularly the formation of the provisional matrix that occurs early during treatment, could compromise the entire process [90]. Furthermore, disruption of the remodeling of this extraction site by osteoclasts, which normally occurs via an accelerated rate of modeling and remodeling [55, 93], may potentially play into the lack of healing that is a prominent feature of BRONJ. The sole evidence on this topic comes from an in vivo study in mice which showed that bisphosphonates did not affect angiogenesis associated with endochondral ossification, a process that is similar to that which occurs with skeletal wound healing [94].

Another aspect related to vasculature, but not as routinely discussed in the BRONJ literature as angiogenesis, is potential effects of bisphosphonates on blood flow [90]. Tissue blood flow is directly proportional to its metabolic activity with the bone receiving ~4-7% of total cardiac output at rest [95] compared to ~17% for skeletal muscle [96]. Blood flow distribution throughout the skeleton is heterogeneous and varies by a factor of ten among bones [97]. Given its high remodeling rate, the mandible would be expected to have high blood flow rates. The lone data concerning mandible blood flow show values for the mandible that are similar to long bones [97, 98]. Importantly, however, these flow rates in the mandible are probably underestimated as the teeth likely account for a significant portion of the mass, yet do not directly receive blood. Regardless of basal blood flow, it would be expected that blood flow to the mandible would be reduced with bisphosphonates due to the suppression of remodeling (which would lower the metabolic demand). This reduced blood flow would lead to vascular remodeling [99] with the skeletal vessels becoming smaller and thus less able to accommodate the demands for skeletal perfusion that are known to exist post-extraction or with infection [100, 101]. The inability to raise blood flow in these circumstances could compromise tissue viability and play a role in BRONJ.

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

Numerous bacteria have been reported in patients with BRONJ yet there is nearly a universal presence of Actinomyces [68, 86, 87 102]. Actinomyces species, most commonly *Actinomyces israelii,* are the most prominent of the over 500 microflora in the oral cavity [103]. Through their formation of a biofilm on the bone/tooth/mucosal surface, Actinomyces perpetuate the adherence of other

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

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

Other hypotheses of BRONJ

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

Future Directions: Data anyone?

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

Figure Legends

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

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

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

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

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

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

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

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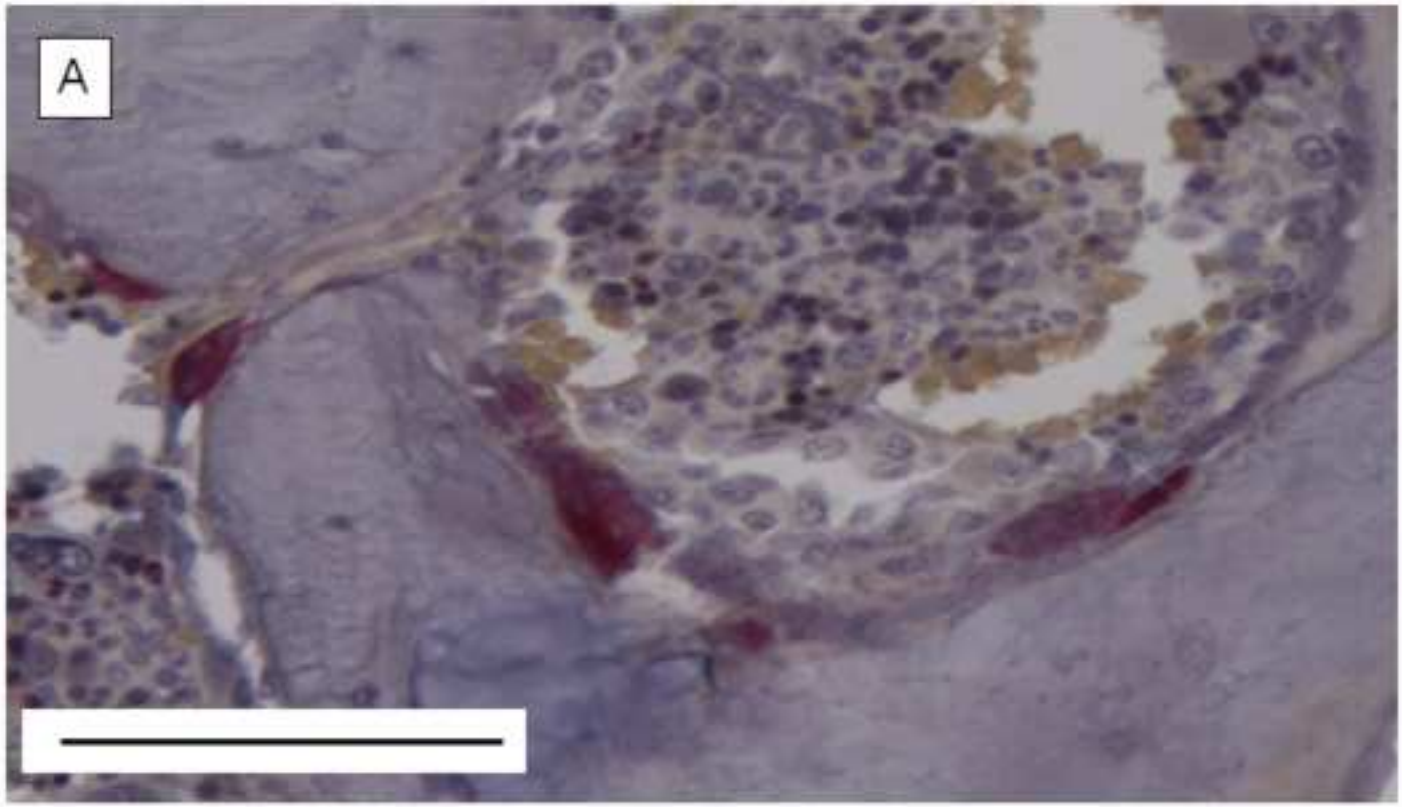

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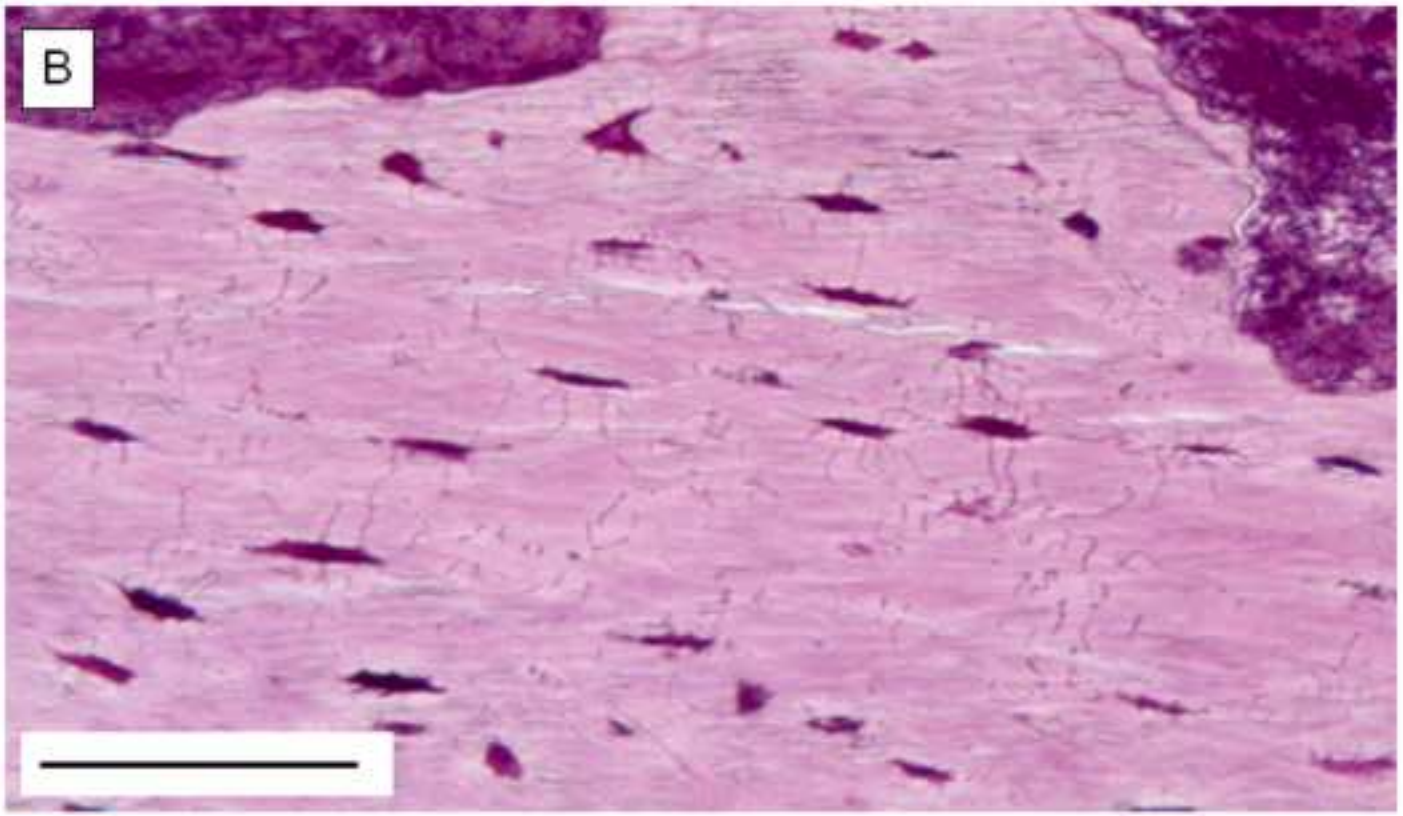

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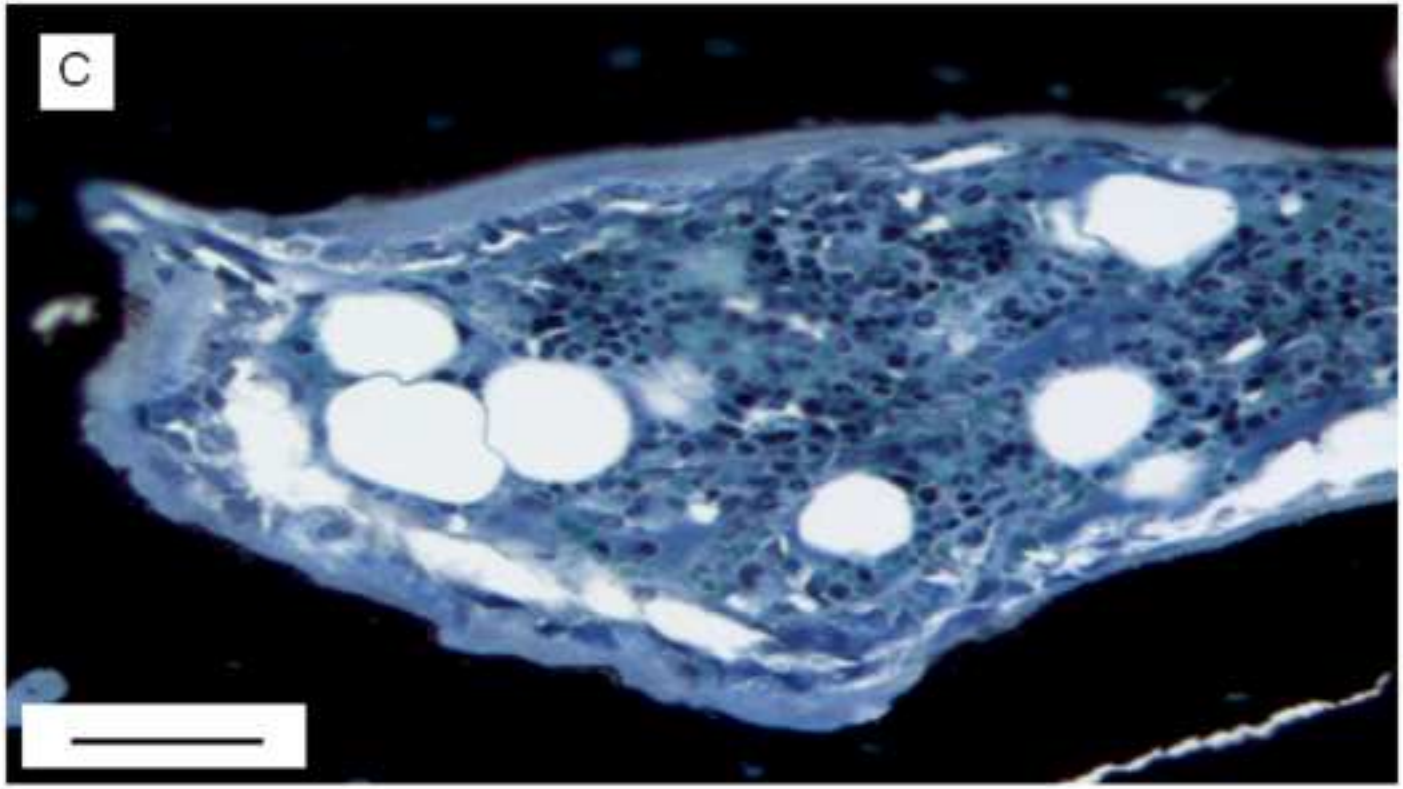

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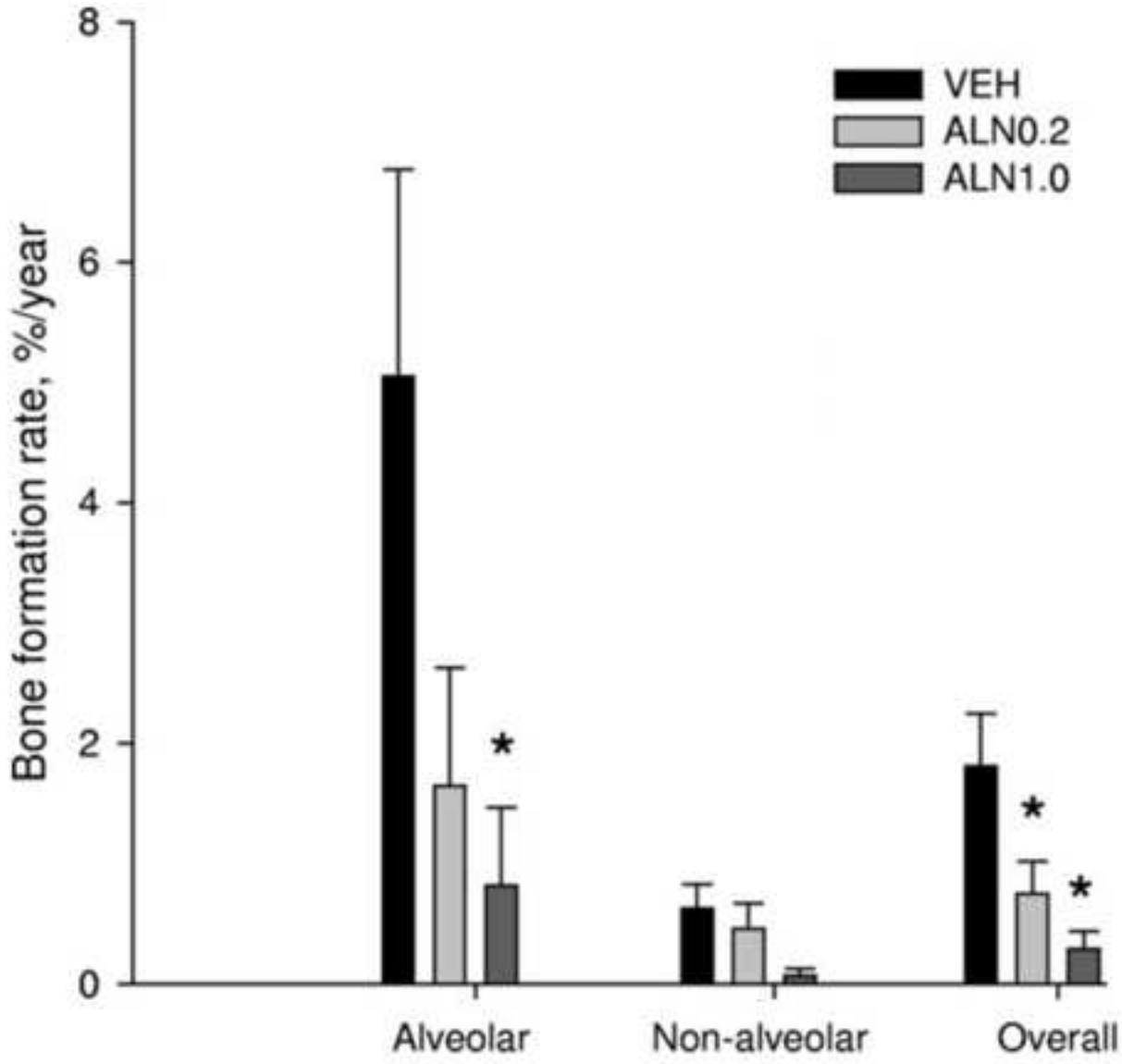

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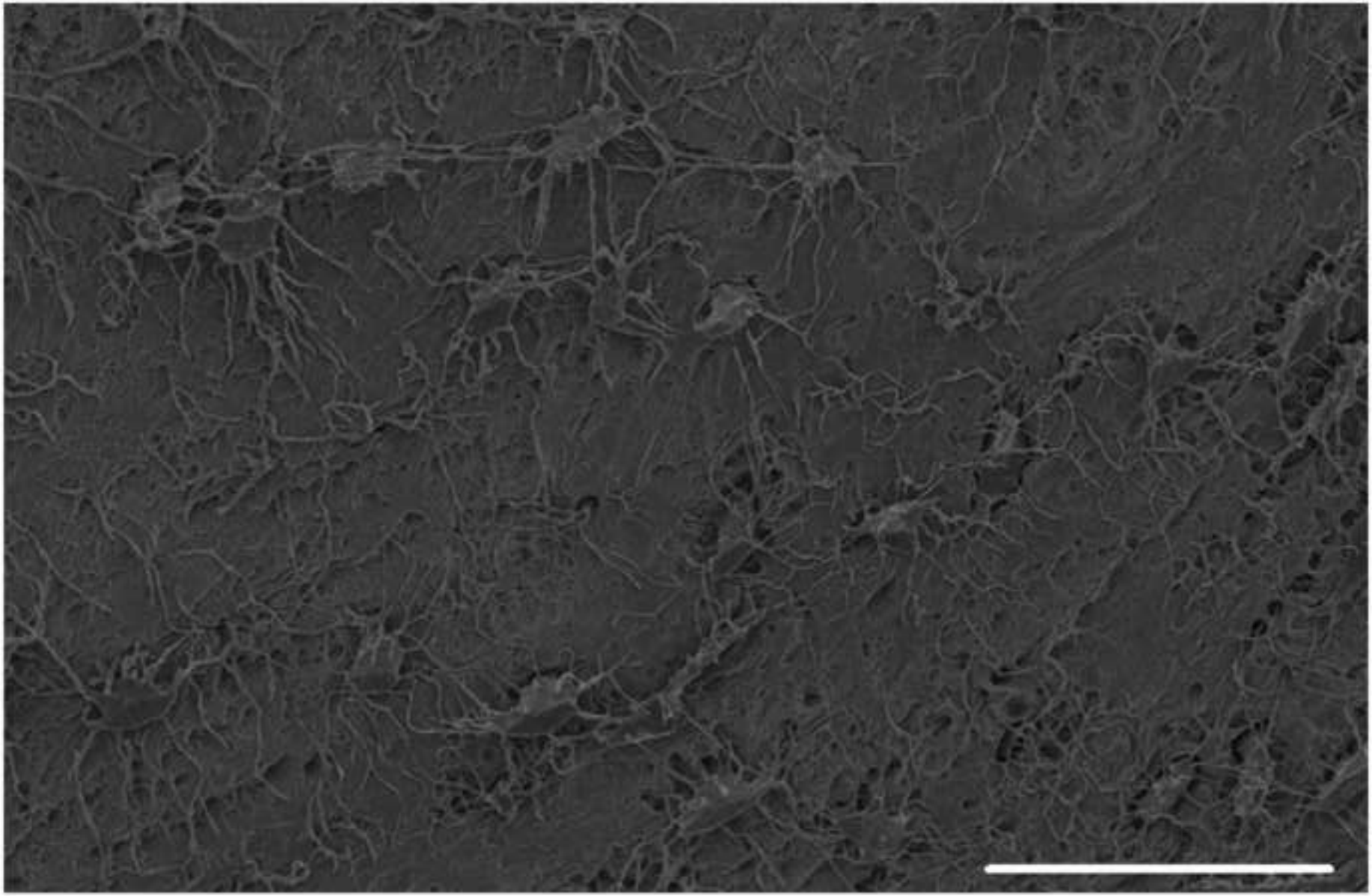

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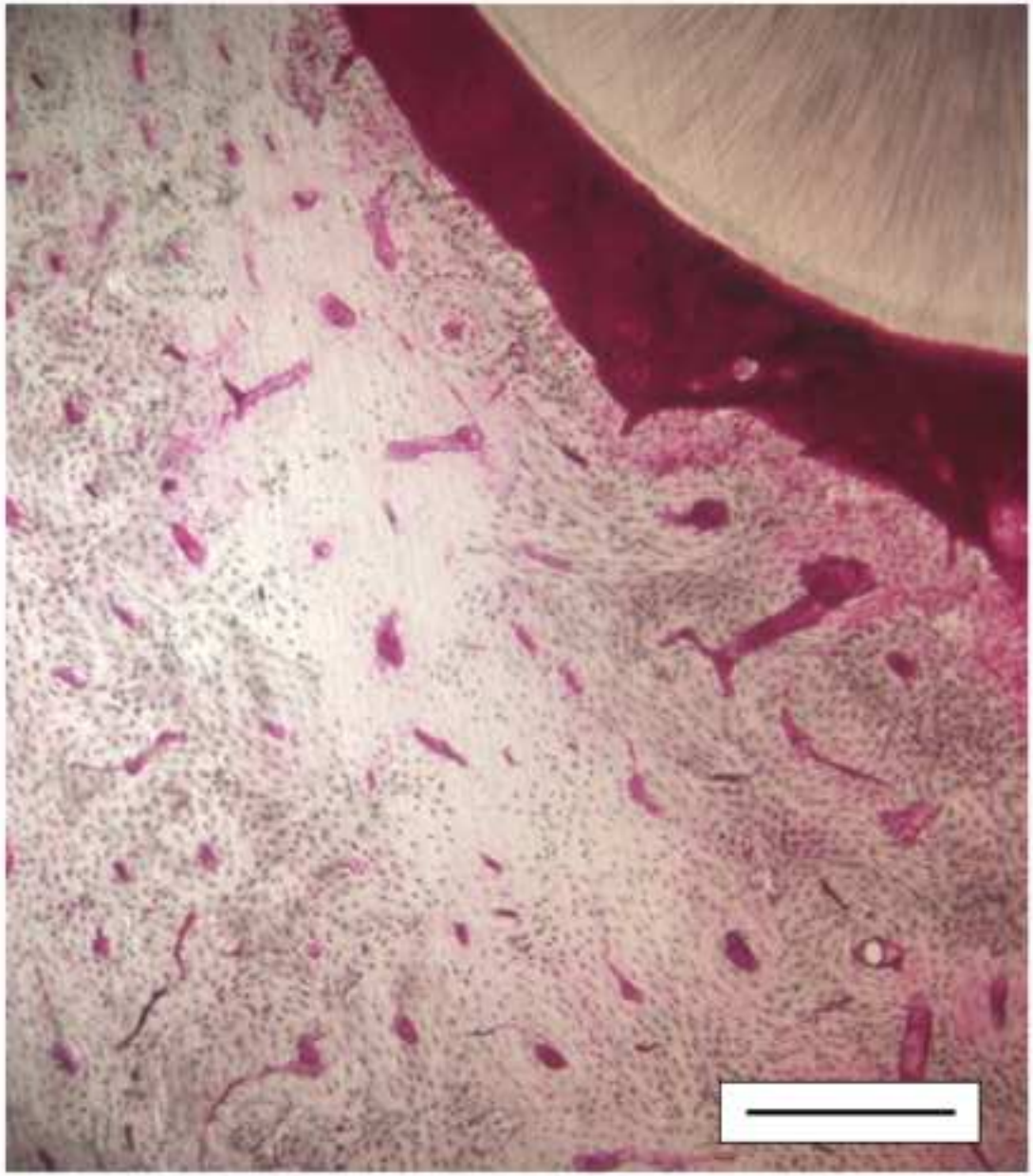

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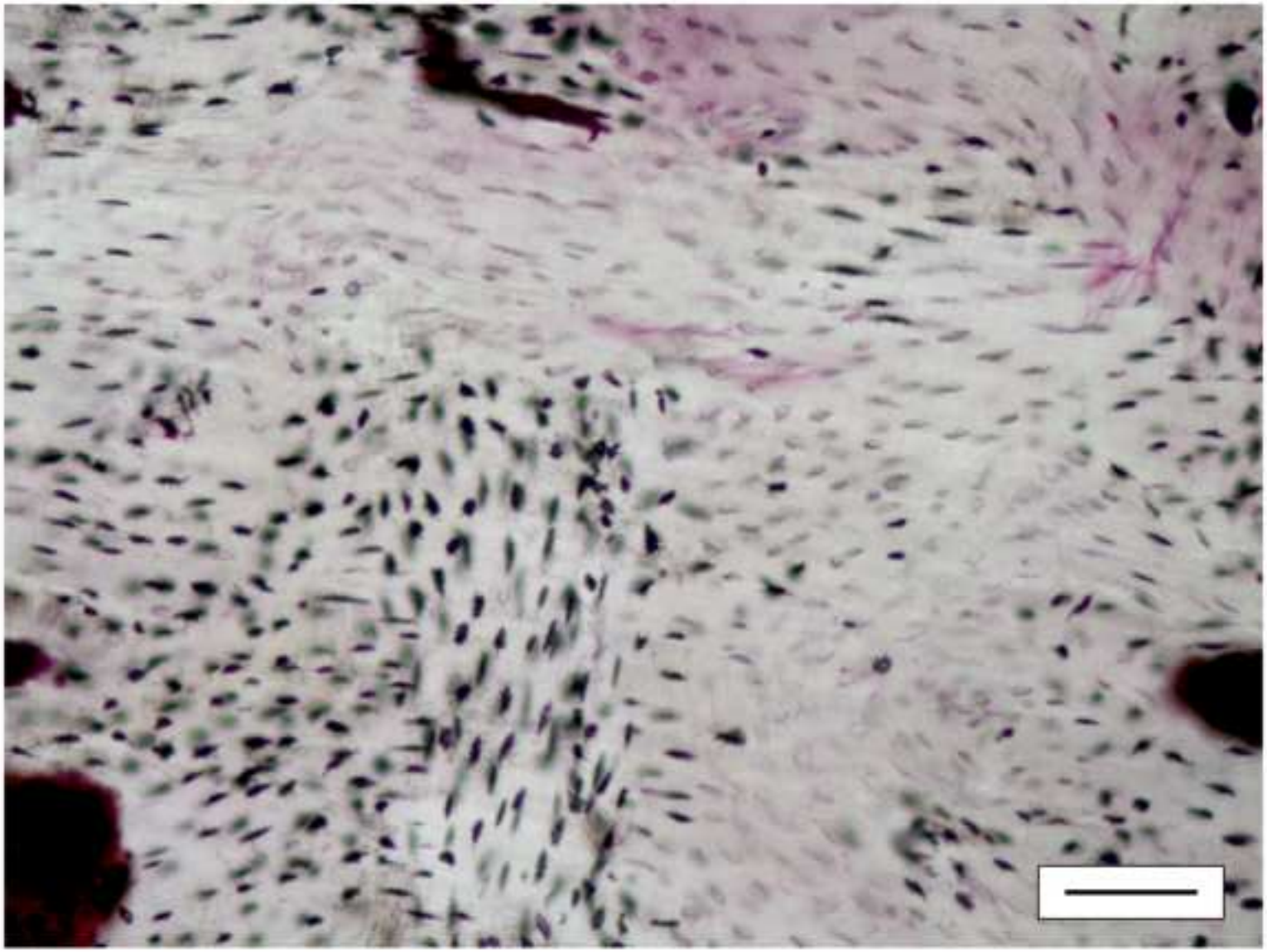

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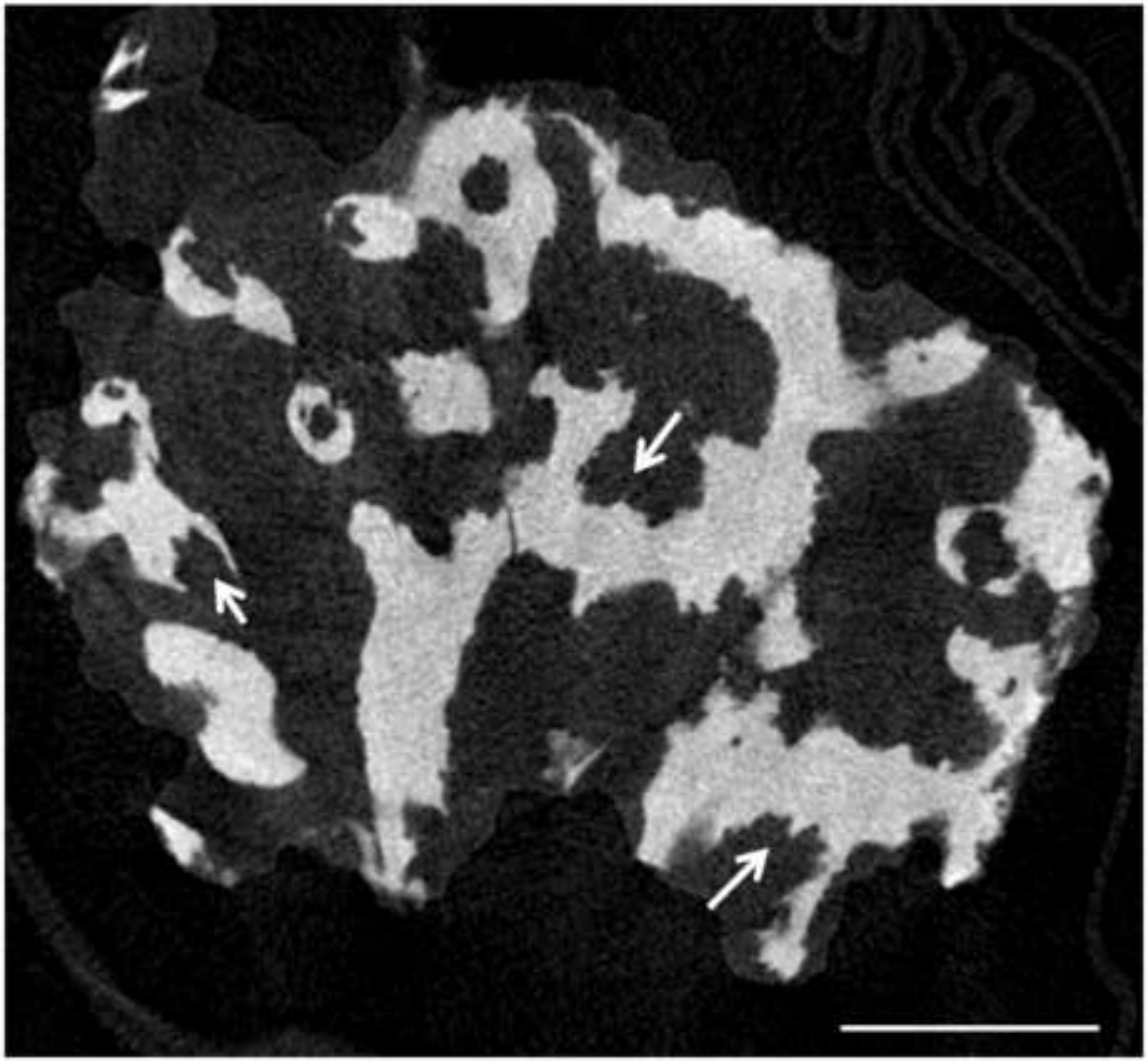

Figure 6