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Animal Studies for Dental Bone Grafting Material Devices - Premarket Notification (510(k)) Submissions

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

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You should submit comments and suggestions regarding this draft document within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <https://www.regulations.gov>. Submit written comments to the Dockets Management Staff, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD 20852. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this document, contact the OHT1: Office of Ophthalmic, Anesthesia, Respiratory, ENT and Dental Devices/DHT1B: Division of Dental and ENT Devices at (301) 796-5620.



**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health**

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Preface

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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff or Office responsible for this guidance as listed on the title page.

I. Introduction

This draft guidance document provides premarket notification (510(k)) submission recommendations for animal studies that may assist manufacturers in complying with some special controls¹ for dental bone grafting material devices. The device is a material that is intended to fill, augment, or reconstruct periodontal or bony defects of the oral and maxillofacial region.² The special controls for dental bone grafting material devices have been set forth in FDA's guidance document, "[Class II Special Controls Guidance Document: Dental Bone Grafting Material Devices](#)" (hereafter referred to as the "Dental Bone Grafting Guidance").^{3,4} The recommendations in this guidance are intended to augment those provided in the [Dental Bone Grafting Guidance](#). The recommendations reflect current review practices and are intended to promote consistency and facilitate efficient review of these submissions.

For the current edition of the FDA-recognized consensus standard(s) referenced in this document, see the [FDA Recognized Consensus Standards Database](#).⁵ If submitting a Declaration of Conformity to a recognized standard, we recommend you include the appropriate supporting documentation. For more information regarding use of consensus standards in regulatory

¹ See 70 FR 21947, available at <https://www.federalregister.gov/d/05-8467>

² 21 CFR 872.3930(a).

³ See 70 FR 22054, available at <https://www.federalregister.gov/d/05-8468>

⁴ <https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/dental-bone-grafting-material-devices-class-ii-special-controls-guidance-industry-and-fda-staff>

⁵ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>

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29 submissions, refer to FDA guidance titled “[Appropriate Use of Voluntary Consensus Standards](#)
30 [in Premarket Submissions for Medical Devices](#).”⁶

31
32 In general, FDA’s guidance documents do not establish legally enforceable responsibilities.
33 Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only
34 as recommendations, unless specific regulatory or statutory requirements are cited. The use of
35 the word *should* in Agency guidances means that something is suggested or recommended, but
36 not required.
37

38 **II. Background**

39 Under sections 513 and 520(l) of the Federal Food, Drug, and Cosmetic (FD&C) Act, FDA
40 published a rule reclassifying tricalcium phosphate granules for dental bone repair from class III
41 (premarket approval) to class II (special controls). Concurrently, the final rule also classified all
42 other bone grafting material devices for dental indications, except those that contained a drug or
43 biologic component, into class II, and revised the classification name and identification of the
44 device type.⁷ The classification identification includes bone grafting materials such as
45 hydroxyapatite, tricalcium phosphate, polylactic and polyglycolic acids, or collagen. Along with
46 this reclassification action, FDA also issued the special controls guidance document [Dental Bone](#)
47 [Grafting Guidance](#), which was finalized April 28, 2005.⁸

48
49 For manufacturers that conduct animal testing, this draft guidance provides animal study
50 recommendations that may help manufacturers satisfy the special control to assess “performance
51 *in vivo*,” as identified under the mitigation measure of “material characterization” in the [Dental](#)
52 [Bone Grafting Guidance](#). Animal studies are generally recommended and provided in premarket
53 submissions for these devices to address the safety and performance *in vivo*, independent of how
54 similar the material and performance characteristics are compared to those of the predicate
55 device(s). Providing an explanation of the history of the safe use of similar devices alone is
56 generally insufficient due to the potential impact of differences in proprietary manufacturing
57 and technological characteristics (e.g., graft shapes and sizes, surface topography, porosity) on
58 the *in vivo* behavior of the bone grafting material devices. As a result, FDA does not
59 recommend extrapolating the *in vivo* behavior of a proposed bone grafting material device from
60 the known *in vivo* behavior of a predicate bone grafting material device. Also, *in vivo* behavior
61 of the bone grafting material typically cannot be adequately evaluated by bench testing methods
62 alone, such as chemical and physical characterizations, because of specific challenges and
63 anatomical differences in replicating the intraoral environment that include, but are not limited
64 to, salivary flow, masticatory forces, food particles, pH and temperature changes, and
65 environment containing unique micro-biota, oral mucosal epithelium and oral musculature. In
66 light of these reasons, FDA is providing additional, detailed animal study recommendations for

⁶ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/appropriate-use-voluntary-consensus-standards-premarket-submissions-medical-devices>

⁷ See 70 FR 21947, available at <https://www.federalregister.gov/d/05-8467>

⁸ <https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/dental-bone-grafting-material-devices-class-ii-special-controls-guidance-industry-and-fda-staff>

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67 these devices to assist manufacturers in providing adequate animal study data, when an animal
68 study is conducted to support a 510(k) submission for dental bone grafting material devices.
69

70 The animal study recommendations in this draft guidance are intended to supplement, and not
71 supersede, the recommendations provided in the FDA guidance “[General Considerations for](#)
72 [Animal Studies Intended to Evaluate Medical Devices](#).”⁹ This guidance document supplements
73 other FDA documents regarding certain content requirements and recommendations of a
74 premarket notification (510(k)) submission.¹⁰
75

76 III. Scope

77 The scope of this draft guidance is limited to animal study recommendations for certain dental
78 bone grafting material devices, which may help manufacturers comply with some of the special
79 controls for these devices. This guidance also includes recommendations to help manufacturers
80 comply with special controls related to biocompatibility assessment of these devices, should the
81 manufacturer choose to combine an animal study to evaluate *in vivo* performance with the
82 biocompatibility evaluation of the implantation endpoint (or the local effects after implantation).
83 The remaining special controls identified in the [Dental Bone Grafting Guidance](#) are outside the
84 scope of this guidance.
85

86 The devices included within the scope of this guidance are limited to the class II bone grafting
87 material devices regulated under 21 CFR 872.3930 with the product codes listed in the table
88 below.
89

90 **Table 1: Applicable Product Codes**

Product Code	Product Code Name	Regulation Number
LYC	Bone Grafting Material, Synthetic	21 CFR 872.3930
NPM	Bone Grafting Material, Animal Source	21 CFR 872.3930
NUN ¹¹	Bone Grafting Material, Human Source	21 CFR 872.3930

91
92 The scope of this guidance does not include the following products:
93

⁹ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-considerations-animal-studies-intended-evaluate-medical-devices>

¹⁰ See 21 CFR 807.87 and the FDA guidance document “Electronic Submission Template for Medical Device 510(k) Submissions” available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/electronic-submission-template-medical-device-510k-submissions>

¹¹ The scope of this guidance includes human demineralized bone matrix (DBM) that is more than minimally manipulated or modified with additives (except for sterilizing, preserving, or storage agents). For more information, please also see the Federal Register notice of January 19, 2001 ([66 FR 5447](#)) and the FDA webpage, “Jurisdictional Update: Human Demineralized Bone Matrix,” available at <https://www.fda.gov/composition-products/jurisdictional-updates/jurisdictional-update-human-demineralized-bone-matrix>

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- 94 • bone grafting materials that contain a drug that is a therapeutic biologic, such as bone
95 morphogenic proteins and other biological response modifiers, under the product codes
96 NPZ and NQA;
97
- 98 • human demineralized bone matrix (DBM), whether minimally manipulated¹² or modified
99 with additives that are sterilizing, preserving, or storage agents; and
100
- 101 • bone grafting materials for non-oral/maxillofacial indications, e.g., for spinal and other
102 orthopedic applications.
103

104 **IV. 510(k) Submission Recommendations**

105 The sections below provide recommendations on animal study information and data to include in
106 a 510(k) submission for dental bone grafting material devices. FDA believes that the animal
107 study recommendations in this draft guidance provide at least the same level of protection of the
108 public health and safety as the animal testing details contained in the [Dental Bone Grafting](#)
109 [Guidance](#). To the extent the recommendations in the following sections depart from previously
110 issued recommendations in the above guidance document, this section supersedes those previous
111 recommendations.
112

113 **A. Animal Studies**

114 An animal study conducted for dental bone grafting materials should address factors that cannot
115 be evaluated through bench tests or in a clinical study. The study design and endpoints should be
116 based upon the mechanism of action of the device and mitigation of identified risks to health. We
117 recommend that your animal study includes the relevant information described in the FDA
118 guidance document “[General Considerations for Animal Studies Intended to Evaluate Medical](#)
119 [Devices](#).”¹³
120

121 FDA supports the principles of the “3Rs,” to replace, reduce and/or refine animal use in testing
122 when feasible. We encourage sponsors to consult with us if they wish to use a non-animal testing
123 method they believe is suitable, adequate, validated, and feasible. If you are proposing to use a
124 non-animal testing method in lieu of an animal study, we recommend that you discuss the
125 proposal using the Q-Submission Program.¹⁴ We will consider if such an alternative method
126 could be assessed for equivalency to an animal study.
127

¹² See 21 CFR 1271.3(f).

¹³ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-considerations-animal-studies-intended-evaluate-medical-devices>

¹⁴ For details on the Q-Submission Program, refer to the guidance “Requests for Feedback and Meetings for Medical Device Submissions: The Q-Submission Program” available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/requests-feedback-and-meetings-medical-device-submissions-q-submission-program>

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128 We also encourage manufacturers to take advantage of the Q-Submission Program to help
129 ensure that the animal study protocol addresses safety and performance concerns and contains
130 elements that are sufficient to support a 510(k) submission.
131

(1) Animal Model

133 Your choice of animal model should be justified. We recommend the use of skeletally mature
134 canine or porcine models, over rodent models, for studying the *in vivo* performance of dental
135 bone grafting material devices. Canine and porcine models are recommended since the dental
136 anatomy of dogs and pigs more closely resemble human dentoalveolar architecture than that of
137 smaller animals.^{15,16,17,18} Moreover, rodents experience continuous bone growth throughout their
138 lifetime,^{19,20} which FDA believes would hamper proper assessment of devices intended to form
139 bone over time. Also, rodents are too small to allow for placement of a sufficient amount of graft
140 material in an intraoral defect site, particularly for resorbable bone grafting material devices that
141 contain granular components. In contrast, periodontal tissues and the size of the teeth in dogs or
142 pigs are, in general, similar to those in humans.
143

144 An animal model should be representative of the full scope of the proposed indications for use by
145 performing studies using anatomical sites consistent with the intended location of use or worst-
146 case defect that covers the scope of indications sought, e.g., intraoral mandibular or maxillofacial
147 models. When a device is intended to be used in an intraoral environment, there are specific
148 challenges and anatomical differences, such as salivary flow, masticatory forces, food particles,
149 pH and temperature changes, environment containing unique micro-biota, oral mucosal
150 epithelium and oral musculature.^{21,22,23} These challenges are significantly different from other
151 bone-associated environments within the human anatomy, e.g., cranial/calvarial or orthopedic
152 applications. As such, cranial/calvarial or orthopedic animal studies are not generally sufficient
153 to support the *in vivo* performance of dental bone grafting material devices.

¹⁵ Dard, M. (2012). Animal models for experimental surgical research in implant dentistry. In Ballo, A. (Ed.), *Implant Dentistry Research Guide: Basic, Translational and Clinical Research* (pp. 167-190). Hauppauge, NY: Nova Science Publishers, Inc.

¹⁶ Dard, M. (2012). Methods and interpretation of performance studies for dental implants. In Bourtrand, J.P. (Ed.), *Biocompatibility and Performance of Medical Devices* (pp. 308-344). Sawston, United Kingdom: Woodhead Publishing.

¹⁷ Wancket, L. M. (2015). Animal models for evaluation of bone implants and devices: Comparative bone structure and common model uses. *Veterinary Pathology*, 52(5), 842-850.

¹⁸ Kantarci, A., Hasturk, H., & Van Dyke, T. E. (2015). Animal models for periodontal regeneration and peri-implant responses. *Periodontology 2000*, 68(1), 66–82.

¹⁹ Pellegrini, G., Seol, Y. J., Gruber, R., & Giannobile, W. V. (2009). Pre-clinical models for oral and periodontal reconstructive therapies. *Journal of Dental Research*, 88(12), 1065–1076.

²⁰ Struillou, X., Boutigny, H., Soueidan, A., & Layrolle, P. (2010). Experimental animal models in periodontology: A review. *The Open Dentistry Journal*, 4, 37-47.

²¹ van der Bilt, A., Engelen, L., Pereira, L. J., van der Glas, H. W., & Abbink, J. H. (2006). Oral physiology and mastication. *Physiology & Behavior*, 89(1), 22–27.

²² Deo, P. N., & Deshmukh, R. (2019). Oral microbiome: Unveiling the fundamentals. *Journal of Oral and Maxillofacial Pathology : JOMFP*, 23(1), 122–128.

²³ Kantarci, A., Hasturk, H., & Van Dyke, T. E. (2015). Animal models for periodontal regeneration and peri-implant responses. *Periodontology 2000*, 68(1), 66–82.

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(2) Study Design Considerations

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a. **Sample Size and Animal Characteristics:** To demonstrate substantial equivalence, the animal study should include a sufficient number of animals to establish trends and to account for potential loss of animals during the course of the study. We recommend a minimum of 3 animals per treatment per evaluation time point. The animal study should be conducted on a minimum (3) samples for each treatment group per time point. The test article should be the device in its final finished form.²⁴ The animal study final report should include animal model information describing the age, sex, breed, and weight of the animals. Additionally, information describing how you have ensured the study animals have reached skeletal maturity and applicable supporting information (i.e., X-ray confirmation of growth plate closure or sourcing certificate from purchasing facility) should be included in the submission.

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b. **Control Test Articles:** We recommend that you select a primary predicate device, reference device,²⁵ or autogenous bone graft as a comparator control that is similar with respect to intended use and technological characteristics (e.g., composition, configuration [block, granule, putty]) to the subject device. For example, a bone grafting material device that contains collagen should be compared to another bone grafting material device that contains collagen with a similar intended use. An empty critical size defect (sham) should also be used to incorporate a negative control (see Sections c and d below for more details).

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c. **Worst-Case Scenario:** The animal model selected should be representative of the proposed indications for use under clinically relevant worst-case conditions to demonstrate the *in vivo* performance of the subject device. For example, for many grafting materials intended for use in guided bone regeneration that include indications for “ridge augmentation” or “filling of bone defect after cystectomy” where the defect size may be critically sized, a 1- or 2-wall critical size defect would be most appropriate to cover the full range of indications. However, if the proposed indications for use will be for use “only in extraction sockets,” a critical size defect model may not be necessary. The design of the animal study should also consider the worst-case scenario of the device configuration being used, such as shape, volume, density, largest model size, porosity, or granular size range, if the device is offered in several variations. If one “worst-case” test article cannot be justified as representative of the full family of devices included in the 510(k) submission, more than one test article should be evaluated in the animal study. A

²⁴ For purposes of this guidance, a device in its final finished form includes all manufacturing processes including packaging and sterilization, if applicable.

²⁵ The definitions for “primary predicate device” and “reference device” are found in FDA’s guidance “The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]” available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/510k-program-evaluating-substantial-equivalence-premarket-notifications-510k>

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191 justification for the selection of worst-case test article(s) should be included in the 510(k)
192 submission.

193

194 **d. Critical Size Defect:** If the proposed indications for use do not specify a defect size, the
195 defect model for the animal study should be a critical size defect to ensure the full scope
196 of the intended use is assessed by the *in vivo* performance testing conducted. A critical
197 size defect is defined as the smallest size intraosseous wound in a particular bone and
198 species of animal that will not heal spontaneously without intervention within a certain
199 time period.²⁶ With a wide variety of animal models (e.g., canine, porcine) and defect
200 types (e.g., 1-wall, 2-wall) available, the discrete size ranges of a critical size defect may
201 vary. The critical size defect should be validated using an empty sham defect
202 demonstrating that the defect cannot be healed on its own.

203

204 **e. Periosteum:** Since the periosteum can influence healing within the bone defect,
205 manufacturers should state whether or not the periosteum has been removed in the animal
206 study final report. The presence or absence of the periosteum within all bone defect sites
207 evaluated in each animal study should be the same to allow for consistent comparison
208 across all evaluation groups (i.e., bone grafting device treatment samples, control test
209 articles, sham defects).

210

211 **f. Healing Period:** For defect models that involve extraction of teeth, such as the intraoral
212 mandibular defect model, we recommend an adequate healing period following tooth
213 extraction (e.g., 3-6 months) before creating the defect. The allowance for a sufficient
214 healing period prior to defect creation ensures that the host bone remodeling has reached
215 a steady/stable state,²⁷ which creates a consistent and homogenous defect model across
216 test sites.

217

218 **g. Study Duration:** Bone grafting material devices resorb and remodel at different rates *in*
219 *vivo*. Therefore, we recommend that each animal study includes a minimum of 3
220 evaluation time points (e.g., 4, 8, and 12 weeks post-implantation). Inclusion of several
221 time points allows for an assessment in trends for graft resorption and new bone
222 formation over time, as well as any inflammatory reactions. The earliest time point (e.g.,
223 4 weeks) allows for an assessment of the initial biologic responses to the device. The
224 intermediate time point (e.g., 8 weeks) should establish interim device behavior between
225 earlier and later time points, as well as demonstrate a reduction of any initial
226 inflammatory response. The final time point (e.g., 12 weeks) should be of sufficient
227 duration to demonstrate bone healing and the effects of any residual device material. For
228 most bone grafting material devices, FDA understands that the final time point may not

²⁶ For the purposes of this guidance, the definition for “critical size defect” is found in the FDA recognized standard ASTM F2721 *Standard Guide for Pre-clinical In Vivo Evaluation in Critical Size Segmental Bone Defects*, which contains information relevant to the design of critical size defect models for the evaluation of bone grafting materials. However, if using this standard, the differences between critical size defect for segmental bone and non-segmental bone should be considered to the specific dental applications.

²⁷ Kenkre, J. S., & Bassett, J. (2018). The bone remodelling cycle. *Annals of Clinical Biochemistry*, 55(3), 308–327.

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229 allow for complete device resorption, but instead, the final time point should demonstrate
230 a trend towards complete device resorption.

231
232 We recommend that bone grafting material devices that contain components that resorb
233 faster than native bone growth and/or are intended to elicit an early healing response
234 should be evaluated at an earlier time point (e.g., 2 weeks). Furthermore, devices that
235 contain slow resorbing materials (e.g., hydroxyapatite) should be evaluated at a later time
236 point (e.g., 26 weeks). The inclusion of such time points for the evaluation of early and/or
237 later device responses (e.g., 2 weeks and/or 26 weeks) is often either incorporated into
238 the 3 evaluation time points recommended above (e.g., 4, 12, and 26 weeks) or added as
239 additional evaluation time points (e.g., 2, 4, 8, and 12 weeks or 4, 8, 12, and 26 weeks) in
240 the animal study. The selected time points and study duration should be justified based on
241 the expected healing response and resorption profile of the bone grafting material devices
242 to allow for a comprehensive assessment of the biological and performance
243 characterizations of the device at relevant time points.

244 **h. Radiography, Histology, and Histomorphometry:** The animal study final report should
245 include the radiographic, histologic, and histomorphometric data to assess bone
246 formation, device resorption, presence of residual material, and generation of degradation
247 particulates or byproducts, if present, at relevant intervals over the duration of healing.
248 Furthermore, the data from radiography, histology, and histomorphometry assessments
249 can demonstrate the quality of the newly formed bone in its ability to support
250 biomechanical loading for the intended use of the device under physiologically-relevant
251 conditions.^{28,29} Therefore, FDA believes that radiography, histology, and
252 histomorphometry data is generally sufficient to demonstrate adequate biomechanical
253 properties of the newly formed bone, without direct biomechanical testing on explanted
254 tissue samples from the defect sites over the evaluation time points.

255
256 For radiography, histology, and histomorphometry assessments, images should be
257 provided for each evaluation time point in an appropriate format, i.e., histologic and
258 histomorphometric images in color with appropriate labels that identify the magnification
259 power, defect area, new bone formation, surrounding bone, test and control articles, and
260 all cell types present. Images from several magnifications should be included (low and
261 high magnification at a minimum). We recommend that manufacturers also consider the
262 following recommendations for how to conduct assessments for radiography, histology,
263 and histomorphometry:

264
265 i. Radiographic image analysis techniques should be used to provide an overall,
266 high level, non-destructive assessment of bone formation, graft resorption,
267 device/graft location, and device/graft migration. To provide useful information
268 concerning the behavior of bone grafting materials in defect sites, radiographic

²⁸ Padiál-Molina, M., Marchesan, J., Taut, A., Jin, Q., Giannobile, W., & Rios, H. (2012). Methods to validate tooth-supporting regenerative therapies. *Odontogenesis: Methods and Protocols*, vol. 887, 135-148.

²⁹ Pellegrini, G., Seol, Y. J., Gruber, R., & Giannobile, W. V. (2009). Pre-clinical models for oral and periodontal reconstructive therapies. *Journal of Dental Research*, 88(12), 1065–1076.

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269 images should be of sufficient quality to allow for discrimination between bone
270 (native, autograft, and newly-formed) and radiopaque bone grafting materials
271 devices. Additionally, these images should be identified by anatomic orientation
272 and focus on the implantation site.

273 Although plain X-ray alone could be sufficient, we recommend you consider the
274 addition of micro-computed tomography (microCT) for each animal at each
275 evaluation time point within the study because the microCT analysis technique
276 can provide additional three-dimensional (3D) detail and quantitative information
277 on device microarchitecture and tissue ingrowth. If other modalities other than
278 plain film radiographs are used, such as microCT, a validation study should be
279 conducted, or leveraged from existing historical information or literature
280 references, to demonstrate the validity and reliability of the modality prior to use.
281 If including microCT evaluations within the animal study, you should carefully
282 consider how such microCT imaging may be affected by the sample (e.g., device
283 constituent material(s), sample preparation), system hardware/software (e.g.,
284 image acquisition parameters, image processing procedures), and methods used
285 for microCT image analysis. The segmentation process is a critical step that can
286 affect the interpretability and validity of microCT results, and we recommend that
287 you justify your segmentation technique in the animal study final report.
288

289 To ensure that microCT results are consistent and comparable across each animal
290 and across evaluation time points, the same scanning protocol should be used for
291 all evaluated samples. We also recommend providing the following additional
292 details in your animal study final report for microCT evaluations conducted
293 during the animal study:

- 294
- 295 a) Description of the microCT instrument (system model and any
296 calibration performed) and image acquisition procedures, including
297 sample preparation (sample positioning and use of contrast agents, if
298 any), scanning medium (if scanning samples *ex vivo*), and scan
299 parameters (energy, beam filtration, integration time, isotropic voxel
300 size or in-plane voxel size, and slice thickness for non-isotropic
301 images).
 - 302 b) Description of the image processing procedures, including selection of
303 a region of interest (ROI) (size, shape, and location, including any
304 anatomical landmarks, offsets, or other criteria used), image filtration
305 (description of any filter applied and key filter parameters), image
306 segmentation (method/algorithm/threshold applied for discriminating
307 between bone and device),³⁰ and correction or reduction of image

³⁰ Additional information on segmentation techniques used in various imaging modalities can be found in the following FDA-recognized consensus standards: (1) ASTM F2603 *Standard Guide for Interpreting Images of Polymeric Tissue Scaffolds* and (2) ASTM F3259 *Standard Guide for Micro-computed Tomography of Tissue Engineered Scaffold*.

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- 308 artifacts (e.g., beam-hardening artifacts, ring artifacts, partial volume
309 effects).
- 310 c) For any quantitative analyses, a description of the image analysis
311 procedures, including the metrics assessed (e.g., bone volume fraction,
312 bone microstructural organization, bone mineral density, tissue
313 mineral density) and the algorithms used.
- 314 d) The method for selecting the locations of the image slices used for
315 analysis within the samples should be justified and should demonstrate
316 consistency across samples. To facilitate visualization of the results,
317 symbols or markers should be used, as appropriate, to highlight key
318 features (e.g., bone growth, device material).
- 319
- 320 ii. Histologic analysis is used to provide a qualitative analysis of the types of tissues
321 present and confirm the presence of bone and residual implant throughout the
322 defect over time. We recommend your animal study final report contains a
323 description of the methods used to prepare the tissues for analysis, including
324 fixation, sectioning, staining, and examination protocols (e.g., manual quantitative
325 methods or automated software). The number of sections per animal and their
326 location within the defect should be explicitly identified. Multiple stains (e.g.,
327 Hematoxylin and Eosin, Masson's Trichrome) can be used to ensure that you
328 capture and identify all tissue types present in the samples. High quality color,
329 digital macro- and micro-photographs should accompany the board-certified
330 veterinary pathologist's report. The purpose of the images is to provide supporting
331 photo documentation of the veterinary pathologist's observations and narratives.
332 We recommend that you include relevant representative sample images from all
333 study animals, which includes photos of the examined device *in situ* and a
334 description of any findings, and an explanation of how bias was avoided in the
335 pathological evaluation of the animal study (e.g., use of blinded procedures, peer
336 review, pre-defined acceptance criteria) when evaluating the tissue reaction to
337 each material and each sample.
- 338
- 339 We recommend including in your 510(k) submission the following in the animal
340 study final report for histological evaluation:
- 341
- 342 a) The comparator and negative (sham) control images. The comparator
343 control article should elicit a known/acceptable tissue response. The
344 sham defect (negative control) should demonstrate that the defect has
345 not healed naturally on its own.
- 346 b) The analysis should be representative of an average of multiple slices
347 obtained at different levels throughout the sample. We recommend a
348 minimum of 3 sections per defect, which are representative of the
349 entire defect area. Each photomicrograph image should include
350 defined symbols (e.g., arrows, asterisks) that clearly highlight critical
351 structures and areas of interest. The margins of the samples should be
352 marked and described in the histological sections examined. The

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- 353 animal study final report should include a description characterizing
354 histopathological changes, such as (but not limited to) fibrosis,
355 inflammation, neovascularization, new bone formation, and presence
356 of device material.
- 357 c) There are advantages and disadvantages associated with the use of
358 decalcified versus non-decalcified histological techniques. A
359 justification for the decalcified technique selected should be included
360 in the animal study final report. We recommend the animal study final
361 report and/or 510(k) submission include a justification for the sample
362 preparation technique selected and an explanation for how the
363 technique allows for the identification of both newly formed and pre-
364 existing bone.
- 365 d) In addition to the visual assessment of new bone formation or device
366 resorption by histological evaluation, and as a complementary method
367 to other performance evaluations (e.g., X-ray, microCT), a
368 comprehensive quantitative method is also recommended, such as a
369 histomorphometry evaluation technique. See additional
370 histomorphometry recommendations in Section IV.A.(2).h.iv. below.
371 The number of histological sections taken per animal and their
372 locations within each defect should be identified.
- 373
- 374 iii. If microCT imaging is utilized, histologic sections should generally correspond to
375 microCT images sliced at approximately the same plane. Comparison of microCT
376 and histologic analyses allows for a more complete representation of the tissues
377 and materials present within the sample.
- 378
- 379 iv. Histomorphometry is used to provide a quantitative assessment of the extent of
380 bone formation and measurement of the amount of graft material remaining over
381 time. The histomorphometric analysis should be representative of an average of
382 multiple slices obtained at different levels throughout the sample and include an
383 assessment of the presence of inflammatory cells. The quantitative method or
384 process used to distinguish new bone, host bone, fibrous tissue, residual implant,
385 and void space on representative histomorphometry images should be described
386 and justified. The region of interest should be clearly defined and exclude any
387 area of host bone. Your histomorphometric analysis should clearly measure the
388 soft tissue formation (fibrous %) in addition to bone formation (bone %) and
389 present the data in the context of the original defect volume/area.
- 390
- 391 v. We recommend that evaluations of resorption assessed in the animal study
392 incorporate the use of baseline measurements taken at Day 0 post-implantation so
393 that the reported results for the planned evaluation time points throughout the
394 study duration (e.g., 4, 8, and 12 weeks post-implantation) can be compared to the
395 initial volume/area of bone grafting materials placed in the defects.
- 396

397 **B. Other Considerations**

398 For manufacturers that choose to combine an animal study that evaluates *in vivo* safety and
399 performance of the dental bone grafting material with a biocompatibility evaluation of
400 implantation (or the local effects after implantation) to help reduce the total number of animals
401 used to support the 510(k) submission, this combined evaluation in the same animal study could
402 be used to partially address the special control for biocompatibility assessment. Specifically, the
403 biocompatibility endpoint of implantation, which is typically conducted per ISO 10993-6
404 *Biological evaluation of medical devices – Part 6: Tests for local effects after implantation* could
405 be combined with the animal study that evaluates *in vivo* performance. Note that manufacturers
406 should separately address the other biocompatibility endpoints listed under ISO 10993-1
407 *Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk*
408 *management process* (e.g., cytotoxicity, sensitization, irritation, genotoxicity) to fully address the
409 biocompatibility of their dental bone grafting material devices.

410
411 If combining the biocompatibility evaluation for the local effects after implantation with the
412 animal study for device performance under one single *in vivo* study, we recommend that you use
413 the methods described in ISO 10993-6 and follow the recommendations in FDA’s guidance “[Use](#)
414 [of International Standard ISO 10993-1, ‘Biological evaluation of medical devices - Part 1:](#)
415 [Evaluation and testing within a risk management process’](#).”³¹ Note that including the
416 biocompatibility assessment for the local effects after implantation within the same intraoral
417 defect animal study intended to evaluate device performance (e.g., assess bone formation, device
418 resorption, presence of residual material, and generation of degradation particulates or
419 byproducts) may necessitate the use of different preparation methods, assessments, and
420 procedures than described in ISO 10993-6 and FDA’s guidance “[Use of International Standard](#)
421 [ISO 10993-1, ‘Biological evaluation of medical devices - Part 1: Evaluation and testing within a](#)
422 [risk management process’](#).”³² We recommend that you provide justifications for the use of any
423 different preparation methods, assessments, and procedures that are modified from ISO 10993-6.
424

425 We recommend submitting a Pre-Submission to discuss any different preparation methods,
426 assessments, and procedures adapted for biocompatibility evaluation of the local effects after
427 implantations within your intraoral defect animal study prior to study initiation. For details
428 regarding Pre-Submissions, refer to the guidance “[Requests for Feedback and Meetings for](#)
429 [Medical Device Submissions: The Q-Submission Program](#).”³³
430

431 For combining an animal study for evaluating device performance and biocompatibility
432 (implantation) endpoints within a single *in vivo* study, we recommend the animal study final
433 report clearly presents each of the assessments for device performance and biocompatibility
434 (implantation) endpoints as separate sections within the animal study final report for clarity. For

³¹ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-international-standard-iso-10993-1-biological-evaluation-medical-devices-part-1-evaluation-and>

³² <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-international-standard-iso-10993-1-biological-evaluation-medical-devices-part-1-evaluation-and>

³³ <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/requests-feedback-and-meetings-medical-device-submissions-q-submission-program>

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435 example, a histological evaluation could be conducted that includes an endpoint defined for
436 animal performance (e.g., bone formation over time, histomorphometry of pre-defined ROI's,
437 lineage-specific stains), as well as the biocompatibility endpoint for the local effects after
438 implantation (i.e., as described in ISO 10993-6). We recommend the animal study final report
439 submitted in the 510(k) submission includes the device performance data and conclusions from
440 the animal study as a separate section from the evaluation of biocompatibility (implantation) data
441 and conclusions. See also Section IV.A.(2).h above for recommendations pertaining to
442 histological and histomorphometry analyses that could be applied to the biocompatibility
443 (implantation) assessment.

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