Contains Nonbinding Recommendations

Draft – Not for Implementation

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Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

Document issued on March 29, 2024.

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draft document within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance.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

Preface

Additional Copies

Additional copies are available from the Internet. You may also send an email request to CDRH-Guidance@fda.hhs.gov to receive a copy of the guidance. Please include the document number GUI00007042 and complete title of the guidance in the request.

Table of Contents

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

Draft Guidance for Industry and Food and Drug Administration Staff

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic.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This draft guidance document provides premarket notification (510(k)) submission recommendations for animal studies that may assist manufacturers in complying with some special controls[1] 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

submissions, refer to FDA guidance titled "Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices."[6]

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

II. Background

Under sections 513 and 520(l) of the Federal Food, Drug, and Cosmetic (FD&C) Act, FDA published a rule reclassifying tricalcium phosphate granules for dental bone repair from class III (premarket approval) to class II (special controls). Concurrently, the final rule also classified all other bone grafting material devices for dental indications, except those that contained a drug or biologic component, into class II, and revised the classification name and identification of the device type.[7] The classification identification includes bone grafting materials such as hydroxyapatite, tricalcium phosphate, polylactic and polyglycolic acids, or collagen. Along with this reclassification action, FDA also issued the special controls guidance document Dental Bone Grafting Guidance, which was finalized April 28, 2005.[8]

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

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

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

III. Scope

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

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

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

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

[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/combination-products/jurisdictional-updates/jurisdictional-update-human-demineralized-bone-matrix

- bone grafting materials that contain a drug that is a therapeutic biologic, such as bone morphogenic proteins and other biological response modifiers, under the product codes NPZ and NQA;

- human demineralized bone matrix (DBM), whether minimally manipulated[12] or modified with additives that are sterilizing, preserving, or storage agents; and

- bone grafting materials for non-oral/maxillofacial indications, e.g., for spinal and other orthopedic applications.

IV. 510(k) Submission Recommendations

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

A. Animal Studies

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

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

[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

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

(1) Animal Model

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

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

(2) Study Design Considerations

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, gender, 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.

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).

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

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

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

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

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

g. Study Duration: Bone grafting material devices resorb and remodel at different rates *in vivo.* Therefore, we recommend that each animal study includes a minimum of 3 evaluation time points (e.g., 4, 8, and 12 weeks post-implantation). Inclusion of several time points allows for an assessment in trends for graft resorption and new bone formation over time, as well as any inflammatory reactions. The earliest time point (e.g., 4 weeks) allows for an assessment of the initial biologic responses to the device. The intermediate time point (e.g., 8 weeks) should establish interim device behavior between earlier and later time points, as well as demonstrate a reduction of any initial inflammatory response. The final time point (e.g., 12 weeks) should be of sufficient duration to demonstrate bone healing and the effects of any residual device material. For 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.

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

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

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

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

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

[28] Padial-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.

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

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

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

a) Description of the microCT instrument (system model and any calibration performed) and image acquisition procedures, including sample preparation (sample positioning and use of contrast agents, if any), scanning medium (if scanning samples *ex vivo*), and scan parameters (energy, beam filtration, integration time, isotropic voxel size or in-plane voxel size, and slice thickness for non-isotropic images).
b) Description of the image processing procedures, including selection of a region of interest (ROI) (size, shape, and location, including any anatomical landmarks, offsets, or other criteria used), image filtration (description of any filter applied and key filter parameters), image segmentation (method/algorithm/threshold applied for discriminating 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*.

artifacts (e.g., beam-hardening artifacts, ring artifacts, partial volume effects).

c) For any quantitative analyses, a description of the image analysis procedures, including the metrics assessed (e.g., bone volume fraction, bone microstructural organization, bone mineral density, tissue mineral density) and the algorithms used.

d) The method for selecting the locations of the image slices used for analysis within the samples should be justified and should demonstrate consistency across samples. To facilitate visualization of the results, symbols or markers should be used, as appropriate, to highlight key features (e.g., bone growth, device material).

ii. Histologic analysis is used to provide a qualitative analysis of the types of tissues present and confirm the presence of bone and residual implant throughout the defect over time. We recommend your animal study final report contains a description of the methods used to prepare the tissues for analysis, including fixation, sectioning, staining, and examination protocols (e.g., manual quantitative methods or automated software). The number of sections per animal and their location within the defect should be explicitly identified. Multiple stains (e.g., Hematoxylin and Eosin, Masson's Trichrome) can be used to ensure that you capture and identify all tissue types present in the samples. High quality color, digital macro- and micro-photographs should accompany the board-certified veterinary pathologist's report. The purpose of the images is to provide supporting photo documentation of the veterinary pathologist's observations and narratives. We recommend that you include relevant representative sample images from all study animals, which includes photos of the examined device *in situ* and a description of any findings, and an explanation of how bias was avoided in the pathological evaluation of the animal study (e.g., use of blinded procedures, peer review, pre-defined acceptance criteria) when evaluating the tissue reaction to each material and each sample.

We recommend including in your 510(k) submission the following in the animal study final report for histological evaluation:

a) The comparator and negative (sham) control images. The comparator control article should elicit a known/acceptable tissue response. The sham defect (negative control) should demonstrate that the defect has not healed naturally on its own.

b) The analysis should be representative of an average of multiple slices obtained at different levels throughout the sample. We recommend a minimum of 3 sections per defect, which are representative of the entire defect area. Each photomicrograph image should include defined symbols (e.g., arrows, asterisks) that clearly highlight critical structures and areas of interest. The margins of the samples should be marked and described in the histological sections examined. The

animal study final report should include a description characterizing histopathological changes, such as (but not limited to) fibrosis, inflammation, neovascularization, new bone formation, and presence of device material.

c) There are advantages and disadvantages associated with the use of decalcified versus non-decalcified histological techniques. A justification for the decalcified technique selected should be included in the animal study final report. We recommend the animal study final report and/or 510(k) submission include a justification for the sample preparation technique selected and an explanation for how the technique allows for the identification of both newly formed and pre-existing bone.

d) In addition to the visual assessment of new bone formation or device resorption by histological evaluation, and as a complementary method to other performance evaluations (e.g., X-ray, microCT), a comprehensive quantitative method is also recommended, such as a histomorphometry evaluation technique. See additional histomorphometry recommendations in Section IV.A.(2).h.iv. below. The number of histological sections taken per animal and their locations within each defect should be identified.

iii. If microCT imaging is utilized, histologic sections should generally correspond to microCT images sliced at approximately the same plane. Comparison of microCT and histologic analyses allows for a more complete representation of the tissues and materials present within the sample.

iv. Histomorphometry is used to provide a quantitative assessment of the extent of bone formation and measurement of the amount of graft material remaining over time. The histomorphometric analysis should be representative of an average of multiple slices obtained at different levels throughout the sample and include an assessment of the presence of inflammatory cells. The quantitative method or process used to distinguish new bone, host bone, fibrous tissue, residual implant, and void space on representative histomorphometry images should be described and justified. The region of interest should be clearly defined and exclude any area of host bone. Your histomorphometric analysis should clearly measure the soft tissue formation (fibrous %) in addition to bone formation (bone %) and present the data in the context of the original defect volume/area.

v. We recommend that evaluations of resorption assessed in the animal study incorporate the use of baseline measurements taken at Day 0 post-implantation so that the reported results for the planned evaluation time points throughout the study duration (e.g., 4, 8, and 12 weeks post-implantation) can be compared to the initial volume/area of bone grafting materials placed in the defects.

B. Other Considerations

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

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

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

For combining an animal study for evaluating device performance and biocompatibility (implantation) endpoints within a single *in vivo* study, we recommend the animal study final report clearly presents each of the assessments for device performance and biocompatibility (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

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