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Reconstruction using Fibula Free Flap

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Corresponding Author: Dr. Nobuhiro Yoda,

Corresponding Author's Institution: Tohoku University Graduate School of
Dentistry

First Author: Nobuhiro Yoda

Order of Authors: Nobuhiro Yoda; Keke Zheng; Junning Chen; Zhipeng Liao;
Shigeto Koyama; Christopher Peck; Michael Swain; Keiichi Sasaki; Qing Li

Abstract: Whilst the newly established biomechanical conditions following mandibular reconstruction using fibula free flap can be a critical determinant for achieving favorable bone union, little has been known about their association in a time-dependent fashion. This study evaluated the bone healing/remodeling activity in reconstructed mandible and its influence on jaw biomechanics using CT data, and further quantified their correlation with mechanobiological responses through an in-silico approach. A 66-year-old male patient received mandibular reconstruction was studied. Post-operative CT scans were taken at 0, 4, 16 and 28 months. Longitudinal change of bone morphologies and mineral densities were measured at three bone union interfaces (two between the fibula and mandibular bones and one between the osteotomized fibulas) to investigate bone healing/remodeling events. Three-dimensional finite element models were created to quantify mechanobiological responses in the bone at these different time points. Bone mineral density increased rapidly along the bone interfaces over the first four months. Cortical bridging formed at the osteotomized interface earlier than the other two interfaces with larger shape discrepancy between fibula and mandibular bones. Bone morphology significantly affected mechanobiological responses in the osteotomized region ($R^2 > 0.77$). The anatomic position and shape discrepancy at bone union affected the bone healing/remodeling process.



東北大学大学院歯学研究科
Tohoku University Graduate School of Dentistry
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575 Japan

Dr. Nobuhiro Yoda
Assistant Professor

Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Tohoku University Graduate School of Dentistry
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575, JAPAN
Telephone: +81-22-717-8369
Facsimile: +81-22-717-8371
Email: nobuhiro.yoda.e2@tohoku.ac.jp

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Dr Richard A Black, Editor-in-Chief
Medical Engineering & Physics

Dear Dr Black,

First of all, we would like to take this chance to thank you for your kind consideration and editorial review of our paper. The editors and the reviewers indeed provided us with highly constructive comments and suggestions, which I believe helped us to make our paper in a much better quality.

Please find the revision of the manuscript and detailed point-by-point responses. We have carefully considered all the comments raised and seriously revised the paper. After many discussion sessions now we all agree to submit you our revision for your further consideration.

Thank you very much again for your consideration of the paper. We look forward to hearing from you at your earliest convenience.

Yours sincerely,

A handwritten signature in black ink that reads 'Nobuhiro Yoda'.

Nobuhiro Yoda, DDS, PhD

Declaration

The authors affirm that this manuscript, entitled “Biomechanical Analysis of Bone Remodeling Following Mandibular Reconstruction using Fibula Free Flap” has been submitted solely to *Medical Engineering & Physics* and that it is not concurrently under consideration for publication in another journal.

The authors also confirm that the submitted work, including images, are original and there is no conflict of interest in this submitted work.

Authors

Signatures

Nobuhiro Yoda



Keke Zheng



Junning Chen



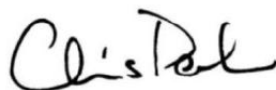
Zhipeng Liao



Shigeto Koyama



Christpher Peck



Keiichi Sasaki



Michael V. Swain



Qing Li



Author Contributions

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data: NY KZ SK KS MS QL.

(2) drafting the article or revising it critically for important intellectual content: NY KZ JC ZL CP KS MS QL.

(3) final approval of the version to be submitted: NY KZ JC ZL SK CP KS MS QL.

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Point-by-point responses to the reviewers' comments on the manuscript

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Dear Editors and Reviewers,

We wish to thank you and expert reviewers for providing the very constructive comments and insightful suggestions on our manuscript. They have helped greatly enhance our manuscript. By closely following your suggestions and incorporating extra information, we hope that the revised manuscript meets the standard of publication for *Medical Engineering & Physics*.

We hope our dedicated revision have addressed all of your concerns. A detailed point-by-point response is provided as follows.

We look forward to hearing from you again at your earliest convenience.

Yours sincerely,

Nobuhiro Yoda, DDS, PhD.



東北大学大学院歯学研究科
Tohoku University Graduate School of Dentistry
4-1 Seiryō-machi, Aoba-ku, Sendai 980-8575 Japan

Biomechanical Analysis of Bone Remodeling Following Mandibular Reconstruction using Fibula Free Flap

Highlights

- The longitudinal changes in bone morphology and mineral density systematically in the course of healing/remodeling after mandibular reconstruction with fibula free flap were quantified;
- The mutual influence between the changes in tissue conditions and mandibular mechanobiology by establishing a combined *in-vivo* and *in-silico* approach was assessed for the first time;
- Novel understanding of mechanobiological responses in a healing and remodeling of mandible following mandibular reconstruction was provided.

1 Biomechanical Analysis of Bone Remodeling Following
2 Mandibular Reconstruction using Fibula Free Flap

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4 Nobuhiro Yoda*^{1,2}, Keke Zheng², Junning Chen³, Zhipeng Liao², Shigeto Koyama⁴,
5 Christopher Peck⁵, Michael Swain⁶, Keiichi Sasaki¹, and Qing Li².

6
7 ¹Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of
8 Dentistry, 4-1, Seiryomachi, Aoba-ku, Sendai, Miyagi, 9808575, Japan

9 ²School of Aerospace, Mechanical and Mechatronic Engineering, The University of
10 Sydney, NSW 2006, Australia

11 ³Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Am
12 Mühlenberg 1 OT Golm, 14476 Potsdam, Germany

13 ⁴Maxillofacial Prosthetics Clinic, Tohoku University Hospital, 1-1, Seiryomachi, Aoba-
14 ku, Sendai, Miyagi, 9808575, Japan

15 ⁵Faculty of Dentistry, The University of Sydney, Sydney, NSW, 2006, Australia

16 ⁶Department of Bioclinical Sciences, Faculty of Dentistry, Kuwait University, Safat
17 13110, Kuwait

18
19 *** Corresponding Author: Nobuhiro Yoda**

20 **Contact details:**

21 Address: Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
22 School of Dentistry, 4-1, Seiryomachi, Aoba-ku, Sendai, Miyagi, 9808575, JAPAN

23 Phone: +81-22-717-8369; Fax: +81-22-717-8371;

24 E-mail: nobuhiro.yoda.e2@tohoku.ac.jp

25 **Abstract**

26 Whilst the newly established biomechanical conditions following mandibular
27 reconstruction using fibula free flap can be a critical determinant for achieving
28 favorable bone union, little has been known about their association in a time-dependent
29 fashion. This study evaluated the bone healing/remodeling activity in reconstructed
30 mandible and its influence on jaw biomechanics using CT data, and further quantified
31 their correlation with mechanobiological responses through an *in-silico* approach. A 66-
32 year-old male patient received mandibular reconstruction was studied. Post-operative
33 CT scans were taken at 0, 4, 16 and 28 months. Longitudinal change of bone
34 morphologies and mineral densities were measured at three bone union interfaces (two
35 between the fibula and mandibular bones and one between the osteotomized fibulas) to
36 investigate bone healing/remodeling events. Three-dimensional finite element models
37 were created to quantify mechanobiological responses in the bone at these different time
38 points. Bone mineral density increased rapidly along the bone interfaces over the first
39 four months. Cortical bridging formed at the osteotomized interface earlier than the
40 other two interfaces with larger shape discrepancy between fibula and mandibular bones.
41 Bone morphology significantly affected mechanobiological responses in the
42 osteotomized region ($R^2 > 0.77$). The anatomic position and shape discrepancy at bone
43 union affected the bone healing/remodeling process.

44

45 **Keywords:** Fibula free flap; Finite element analysis; Jaw biomechanics; Mandibular
46 reconstruction; Bone remodeling.

47

48 **1. Introduction**

49 Free vascularized osteocutaneous tissue transfer has become a well-established
50 procedure for maxillomandibular reconstruction following large resection due to trauma,
51 atrophy, and tumors ablation [1,2]. Fibula free flap (FFF) provides superior length and
52 long vascular pedicles for mandibular reconstruction, with proven subsequent high
53 reliability and adaptability [3]. Nevertheless, some clinical complications remain with
54 delayed or poor union between the grafted fibula bone and host native mandible [4,5].
55 Recent CT evaluations reported 20% [6] and 9% [7] non-union rates, respectively. Bone
56 union determines the strength and health of the reconstructed mandible, both of which
57 are essential for further occlusal and prosthetic rehabilitation. In the case of bone
58 fracture healing, the mechanobiological environment, which is thought to regulate
59 cellular behaviors, can be a critical determinant [8].

60 Unlike general bone fracture healing processes, FFF mandibular reconstruction
61 may be affected by additional factors, such as shape discrepancy between different
62 bones and poor bone vascularity [4,9]. Further, the loss of several masticatory muscles
63 due to resection can cause unbalanced jaw movement and abnormal mastication, leading
64 to significant change in the biomechanical conditions [10,11]. Thus the
65 mechanobiological responses in the jaw can be altered significantly; and such a change
66 in-turn affects subsequent bone remodeling activities [12,13]. To assist surgical planning
67 and oral rehabilitation it is essential to understand bone healing/remodeling activity and
68 its influence on jaw biomechanics, thereby preventing delayed or poor union of bone
69 grafts.

70 Finite element (FE) analysis has the adequacy for the biomechanical studies on

71 orthopaedic [14-16] and dental problems [17-19]. Several those studies demonstrated
72 their compelling advantages for understanding the biomechanics and mechanobiology
73 of reconstructed mandibles *in-silico* [20,21]. With recent advances in micro
74 computerized tomography (CT), bone mineral density (BMD) and morphological
75 changes can be measured to evaluate bone remodeling sequences noninvasively
76 [13,22,23]. The CT-based 3D FE models can be thus created to quantify biomechanical
77 responses to functional forces in a patient-specific and time-dependent manner [24,25].

78 This study aims to (1) examine longitudinal changes in bone morphology and
79 mineral density in the course of healing/remodeling after mandibular reconstruction
80 with FFF; and (2) investigate the associated variation in mandibular biomechanics in
81 terms of mechanical stimulus. The postoperative CT scans were performed at 4 critical
82 time points over two and half years' clinical follow-up, and the CT images were
83 segmented for both 2D multiple planar reconstructions (MPR) and 3D (volumetric)
84 analyses. The bone condition was analyzed in both spatial and temporal manner, in
85 terms of morphology and BMD. Nonlinear 3D FE analyses were conducted to quantify
86 the bone mechanobiological stimuli at these different time points; and then correlated to
87 the corresponding *in-vivo* clinical data. By establishing this combined *in-vivo* and *in-*
88 *silico* approach, the mutual influence between tissue conditions and mandibular
89 mechanobiology was assessed. The results are expected to provide important insights
90 into surgical plan for mandibular reconstruction.

91 **2. Materials and Methods**

92 ***2.1 Clinical Treatment***

93 A 66-year-old male patient received mandibular reconstruction with

94 osteotomized FFF, due to a squamous-cell carcinoma at the right molar gingiva at the
95 Department of Otolaryngology-Head and Neck Surgery, Tohoku University Hospital in
96 Japan. Upon harvesting, the fibular bone was segmented to match the defect jaw
97 morphology. A titanium fixation plate (Synthes, Solothurn, Switzerland), which was
98 pre-bent using the CT-based 3D patient model before surgery, was configured to be
99 fixed monocortically with a total of 11 titanium screws (Synthes, Solothurn,
100 Switzerland) as shown in Fig. 1. The first CT scan (M0) was performed at the end of
101 surgery, and the follow-up CTs were taken at 4, 16 and 28 months after surgery (namely,
102 M4, M16, and M28, respectively). A removable partial denture was inserted into this
103 subject 6 months after the surgery; however, the subject did not use it for mastication,
104 due to fear of biting on the reconstructed side. The periodontal conditions of the
105 remaining teeth and the removable partial denture have been maintained at the
106 Maxillofacial Prosthetics Clinic in Tohoku University Hospital every three months.

107 ***2.2 CT Imaging Acquisition and 2D Image Analysis***

108 Multi-detector helical CT scans were performed for the follow-up examinations
109 using Somatom Emotion 6 (Siemens, Erlangen, Germany) at 120 kV and 80 mA with
110 the spatial resolution of 0.4, 0.4, and 0.8 mm in the radial, tangential, and axial
111 directions. The CT data was further processed with the medical image viewer software
112 (EV Insite S, PSP Co., Tokyo, Japan), for the detection and alignment of anatomic
113 landmarks between the different cross-sectional examinations. The mandibular plane
114 was defined using three reference points; namely, left Gonion point, Menton point, and
115 inflection point of a titanium fixation plate (green triangles in Fig. 2a). Six planes
116 parallel to this mandibular plane were selected for the quantitative analysis of bone
117 union at three docking sites (DS1, DS2, and DS3, respectively) with 2 mm intervals by

118 multiple planar reconstructions (MPR) (Fig. 2b) [12]. On each plane, a 2 mm³ volume
119 of interest (VOI) was considered along the superior-inferior axis (Fig. 2b). Since a
120 significant correlation between Hounsfield units (HU) obtained from clinical CT scans
121 and bone mineral density (BMD) were established [26], the HU values change in VOIs
122 can be regarded as the BMD changes over time here, particularly for bone unification at
123 the contact interfaces. All the VOIs were placed at the same positions throughout these
124 four time points, based on the distance from the titanium fixation plate and screws as a
125 reference.

126 ***2.3 3D Registration and Volumetric Analysis***

127 3D registration was carried out for investigating the longitudinal changes in
128 bone surface profile and mineral density using Amira 2016.22 (Zuse Institute Berlin
129 (ZIB), Berlin, Germany) (Fig. 3a). The titanium fixation plate was selected as the
130 reference geometry for its rigidity and high contrast. To quantify the variation of BMD
131 at the docking sites, the change in greyscale was correlated with the distance from the
132 inferior to the superior aspect. The average value of the pixel intensity (i.e. greyscale)
133 was calculated in the cortical bone region on each slice (at a regular spacing of 0.8 mm
134 along the coronal axis), enabling a plot of pixel value change along the axial direction.
135 To determine the HU values of the cortical bone, several profile lines were constructed
136 at the CT images cross the region of mature cortical bone. By sampling the histogram
137 distribution, a HU value of 1536 was determined to be a threshold for determining
138 cortical bone pixels, which is consistent with the reported HU value of cortical bone for
139 cone beam CT in literature [27]. By using this cortical bone threshold, variation in both
140 bone density and volume at the same region for the four time points were quantified.
141 The detailed variation in bone volume (i.e. volume of the cortical bone voxel cuboids)

142 along this direction was plotted using the same approach. In addition, the variation in
143 pixel number, rather than the pixel intensity, in the cortical bone region was considered.

144 *2.4 Finite Element Analysis*

145 Four case-specific FE models were created based on the CT data taken at M0,
146 M4, M16, and M28, respectively [28,29]. The CT images were imported into ScanIP
147 Ver. 4.3 (Simpleware Ltd, Exeter, UK) for segmentation. The segmented masks (bone,
148 individual tooth and titanium fixation plate) were further processed in Rhinoceros 4.0
149 (Robert McNeel & Associates, Seattle, USA) to create parametric models with non-
150 uniform rational B-spline (NURBS) (Fig. 3b). Following the development of the
151 mandibular models, the total 11 fixation screws were modeled according to the
152 manufacturing specifications in Solidworks 2013 (SolidWorks Corp, Waltham, MA,
153 USA). Those screws were virtually inserted into the models in Rhinoceros 4.0 as guided
154 by the CT images. Considering that the patient disuse the denture in his daily life and
155 has no parafunctional habit, the denture was not inserted in the models. To ensure the
156 numerical accuracy, an adaptive mesh was generated based on a mesh convergence test.
157 Ten-node quadratic tetrahedral elements with hybrid formulation (C3D10H) were
158 adopted to ensure smoothness along the contact interfaces.

159 A pixel-based mapping algorithm was adopted to create the heterogeneous bone
160 density distributions at the different time points, reflecting the changes of the
161 anatomical conditions [29]. A homogeneous isotropic linear-elastic model was used to
162 define the teeth (Young's modulus $E = 20,000$ MPa, Poisson's ratio $\nu = 0.2$), titanium
163 fixation plates and screws (Ti6Al7Nb: $E = 110,000$ MPa, $\nu = 0.3$) [21,30].

164 The hinge constraints were prescribed for the corresponding mandibular
165 condyles. In this subject, the large bone resection was accompanied by the functional

166 loss of the right masseter, medial pterygoid and temporalis muscles; and consequently
167 masticatory conditions changed dramatically post-surgery. Due to lack of information
168 regarding muscular forces after such a large resection [20], the magnitudes and
169 directions of individual forces were derived based on the literature for the remaining
170 muscles (masseter muscle: 59.23 N, medial pterygoid muscle: 39.60 N, lateral pterygoid
171 muscle: 34.44 N, and temporalis: 34.09 N, respectively) [31].

172 Strain energy density (SED) was quantified as a mechanobiological stimulus to
173 analyze the bone responses in the three docking sites and VOIs. SED has been
174 considered an effective stimulus to bone remodeling in long bones [32] and mandible
175 [24,33] and can be a scalar quantity to combine stress and strain but eliminate their
176 directionalities [34]. The SEDs at different time points were correlated with the
177 corresponding change in the bone density. In this study, linear regression analysis was
178 performed using IBM SPSS Statistics Ver. 21.0 (IBM Corp., New York, NY, USA) to
179 examine the correlations between stimuli and bone remodeling progression in all VOIs.
180 The R^2 values presented the goodness of fit for the predictor functions, thereby
181 indicating the extent of correlation.

182 **3. Results**

183 *3.1 MPR Image Assessment for Bone Morphology and Mineral Density*

184 Fig. 4 shows the longitudinal changes in bone profile from the CT-based MPR
185 images. In docking site DS1, a significant amount of callus bone formed at time point
186 M4, and the cortical bridging successfully formed in both buccal and lingual regions at
187 M16. In DS2, the cortical bridging formed at M4 in both the buccal and lingual regions.
188 Also, the cortical-like bone appeared to fill the entire interface, while some resorption

189 occurred at the upper and bottom surfaces of cortical bone. In DS3, there was large
190 discrepancy of bone shape at the initial stage. However, the bone shapes gradually
191 remodeled and cortical bridging was found in both the buccal and lingual regions at
192 M16.

193 Fig. 5 shows that the averaged HU value was calculated for each VOI to
194 quantify the change of BMD. For DS1, both superior and inferior cortical bones
195 underwent resorption from M0 to M16, while the BMD peaked in the trabecular
196 interface regions at M4 before undergoing resorption. In contrast, the grafted bones at
197 DS2 performed exceedingly well in terms of new bone formation, despite being
198 osteotomized, seen in rapid increases of BMD in the first four months. For DS3, the
199 cancellous/trabecular region underwent much more dramatic remodeling than the
200 cortical bone with rapid increase in BMD from M0 to M4 but decrease from M4 to M16.

201 ***3.2 Volumetric Assessment of Bone Mineral Density and Morphology***

202 Bone morphological changes were visualized as the apposition and resorption on
203 the bone surface by 3D volumetric registration in the three docking sites (Fig. 6). The
204 longitudinal changes in bone volume were site-specific and the rate of volume increase
205 in the cortical bone region was positive in all the three sites from M4 to M16 (Fig. 7a).
206 Fig. 7b exhibits the longitudinal change rate of bone volume at each docking site. Bone
207 volume increased remarkably from M4 to M16 due to new bone formation, especially at
208 the region from 15 mm to 25 mm for DS1 and from 20 mm to 30 mm for DS3 on the
209 sectional plane of mandible as visualized in Fig. 6. Fig. 7c plotted the site-specific
210 change rate of BMD based on the average grayscale in the cortical bone region. Note
211 that the BMD decreased in the first four months for all the docking sites.

212 **3.3 Mechanobiological Stimulus Distribution**

213 Fig. 8 shows the longitudinal changes in the SED distribution and corresponding
214 CT MPR images of the reconstructed mandible. Both global and local SED distributions
215 changed with time significantly. The longitudinal changes in morphology and BMD
216 were remarkable particularly for DS1, leading to substantial variation in the SED
217 distribution.

218 The SED at VOIs in the cortical bone region was generally higher than that in
219 the cancellous region in DS1 and DS2 (Fig. 9). At each VOI, the SED decreased with
220 time at DS1 and DS3, especially in the superior region of DS1. While the increase in
221 SED with time could be found in some VOIs, the SED dropped from M0 to M4 and
222 then gradually increased till M28 (but never exceeds that at M0), at 6, 8, and 10 mm
223 VOIs in DS2.

224 Linear regression analysis between the HU values and SED in VOIs indicated
225 that there was a strong dependence on the HU values only in DS2 ($p < 0.05$), as shown in
226 Fig. 10.

227 **4. Discussion**

228 Both 2D MPR images and 3D volumetric analyses enabled to quantify and
229 visualize time-dependent bone apposition and resorption in terms of morphology and
230 BMD in this FFF reconstructive mandible. This study is believed to be the first of its
231 kind for investigating the anatomical sequence of healing/remodeling process and its
232 correlation with mechanobiological responses in a reconstructive mandible.

233 The clinical process of cortical bridging at bone docking regions was found to be

234 significantly site-specific based on the results of both 2D MPR images and 3D
235 volumetric analyses. Biological healing at bone union is influenced by complex cellular
236 and molecular activities, and can be affected by the dimension of bone segment gap [35]
237 and contact shape [9]. In this study, we set up a criterion to justify the cortical bridging,
238 namely, no gap was observed between the two bones in the six cross sectional planes as
239 shown in Fig. 4. According to this criterion, the contact region in DS2 achieved earlier
240 cortical bridging than the other two sites.

241 The BMD became higher within the first four months in all the VOIs except for
242 the cortical bone regions in DS1 (Fig. 5). Those cortical regions appeared to undergo
243 significant resorption, while the osseous callus was generally found at the interface of
244 trabecular regions during the bone-healing phase [36,37]. The BMDs of all the cortical
245 bone regions in the docking sites were also found to decrease in the first four months,
246 which was most remarkable for DS2 (Fig. 7c). Despite a vascularized bone graft, the
247 lower bone vascularity may have caused the reduction of BMD on the cortical region of
248 the fibula graft [38,39]. Despite the lowered BMD, 3D volumetric analysis revealed a
249 higher increase rate of bone volume in DS1 than the other two sites over the same time
250 period (Fig. 7a). Primary bone apposition may have developed throughout formation of
251 the osseous callus at the endochondral and periosteal areas (Figs. 4 and 5) [35]. The
252 woven bone with low BMD appears to initially form for filling the gap and reducing
253 morphological discrepancy, which may be related to the initial volume increase in DS1.
254 Lamellae bone with high BMD appears to form after M4 [37]. Lower bone vascularity
255 in the distal segment of osteotomy [39] may limit those biological healing activities in
256 DS2 and DS3 compared to DS1, further contributing to the initial reduction in the bone
257 volume (Fig. 7a).

258 Considering the positive increase rates attributable to bone apposition at all three
259 docking sites from M4 to M16, bone (re)modeling activity had a primary effect on post-
260 healing bone formation [40,41]. Osseous callus at the interface regions in DS1 and DS3
261 gradually became cancellous bone, forming a natural mandibular structure during the
262 course. Nevertheless, the healing and remodeling process at the docking site, especially
263 with large shape discrepancy, is considered to be significantly slower than those of the
264 general bone fracture [37,42]. Note that the mandible can be distorted during daily oral
265 function [43]. Despite the mechanical fixation by titanium plate, the distortion can affect
266 the mechanical stability of the docking sites, which might also delay the healing process
267 [9].

268 Mechanical loading is known to stimulate bone healing and remodeling process,
269 likely enhancing bone mass and functionality [40]. The mechanobiological impetus can
270 thus be related to the bone remodeling activity [12,13]. SED has been considered an
271 effective stimulus to bone remodeling in long bones [32] as well as mandible [24,33].
272 This study revealed the correlation between SED and healing/remodeling outcome over
273 the time period concerned.

274 The variation in SED distribution was attributed to the time longitudinal change
275 in the mandibular morphology (Figs. 8 and 9), as well as load transfer in the
276 restructured mandible, particularly through the fibula grafts. In other words, the
277 functional load was initially transferred to the fibula graft completely via the titanium
278 fixation plate (M0); but subsequently, a greater proportion of load transferred through
279 the bony tissue as the extent of bone union increased. In addition, the remaining
280 unbalanced muscle activities readapt with time [10,11]. All these factors have a
281 collective effect on the mechanobiological responses.

282 As shown in Fig. 10, the SED had a strong dependence on the HU values in DS2
283 ($p < 0.05$). The HU value altered the load bearing capability of the fibula bone, meaning
284 that the SED is associated with HU values. Lower bone vascularity and good bone
285 contact condition at DS2 possibly enhance the effect of mechanobiological stimuli on
286 BMD adaptation, which might be related to the earlier process of cortical bridging at the
287 DS2. For DS1 and DS3, significant shape discrepancy due to reconstruction generated
288 non-physiological stress/strain concentration, which might have distorted the
289 distribution of SED and its correlation to remodeling.

290 Clinically, the implant-supported denture is considered as the most suitable
291 option for functional rehabilitation following mandibular reconstruction [2]. Although
292 the timing of implant placement is still controversial, several studies adopted the time
293 for implant placement at least 6-12 months after the reconstruction with FFF [1,44,45].
294 Considering the cortical bridging as a predictor of bone union strength [7,46], all the
295 bone unions can be confirmed through CT scanning, especially in the cases with a large
296 bone discrepancy. Specifically, favorable initial bone contacts with small shape
297 discrepancy are considered a primary factor for earlier success of cortical bridging.

298 There are still some limitations in this study. Constrained by the clinical protocol
299 and radiation dosage allowance, the scanning resolution of CTs could have affected
300 modeling accuracy. The FE analyses still included several assumptions, such as
301 simulation under static loading conditions and rotational movement on the mandibular
302 condyles. The applied muscle forces did not precisely reflect specific condition of this
303 subject; plus the muscle forces are anticipated to change over time after reconstruction
304 [47,48]. Consequently, the resultant reaction responses on both temporomandibular
305 joints might become asymmetric and physiologically complicated. Finally, while the

306 study was featured as patient-specific, the results were based on only one particular
307 subject. In addition, other patient's factors, such as the systematic background and the
308 treatment process, could be generally the decisive factors to the bone healing and
309 remodeling process at the docking sites. Further evaluation and data acquisition of other
310 subjects with inevitably varied conditions are necessary before generalizing these
311 clinical and biomechanical findings.

312 **5. Conclusion**

313 This newly developed analysis procedure provided a quantitative clinical follow-
314 up of mandibular reconstruction with fibula free flap (FFF) and fundamental
315 understanding of time-dependent biomechanical responses in the reconstructed
316 mandible. It was found that the bone healing and remodeling process at the docking
317 sites were site-specific; and cortical bridging in the osteotomized region took place
318 faster than that in the other docking sites between mandibular and fibula bones for the
319 specific patient concerned. Within the limitation of this study, the anatomic position and
320 the discrepancy of initial shape at the docking sites between the host mandible and
321 fibula graft affected the bone healing and remodeling process. It divulged a correlation
322 between mechanobiological stimulus (strain energy density - SED) and the longitudinal
323 change in bone mineral density (BMD) and morphology, especially at the osteotomized
324 region. The longitudinal CT data and mechanobiological correlation generated in this
325 study provided new insights into patient-specific surgical planning and occlusal
326 rehabilitation.

327

328 **Conflict of interests**

329 None declared.

330

331 **Ethical approval**

332 The research protocol was approved by the research ethics committee of the Tohoku
333 University Graduate School of Dentistry (reference #24-10). Full written informed
334 consent was obtained to use the CT images for this study. All procedures performed in
335 this study were in accordance with the ethical standards of the 1964 Helsinki
336 Declaration (<http://www.wma.net>) and its later amendments.

337

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340 Discovery and Fellowship schemes (DP160104602 and FT120100947). We are grateful
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343

344 **References**

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1 Biomechanical Analysis of Bone Remodeling Following
2 Mandibular Reconstruction using Fibula Free Flap

3
4 Nobuhiro Yoda*^{1,2}, Keke Zheng², Junning Chen³, Zhipeng Liao², Shigeto Koyama⁴,
5 Christopher Peck⁵, Michael Swain⁶, Keiichi Sasaki¹, and Qing Li².

6
7 ¹Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of
8 Dentistry, 4-1, Seiryomachi, Aoba-ku, Sendai, Miyagi, 9808575, Japan

9 ²School of Aerospace, Mechanical and Mechatronic Engineering, The University of
10 Sydney, NSW 2006, Australia

11 ³Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Am
12 Mühlenberg 1 OT Golm, 14476 Potsdam, Germany

13 ⁴Maxillofacial Prosthetics Clinic, Tohoku University Hospital, 1-1, Seiryomachi, Aoba-
14 ku, Sendai, Miyagi, 9808575, Japan

15 ⁵Faculty of Dentistry, The University of Sydney, Sydney, NSW, 2006, Australia

16 ⁶Department of Bioclinical Sciences, Faculty of Dentistry, Kuwait University, Safat
17 13110, Kuwait

18
19 *** Corresponding Author: Nobuhiro Yoda**

20 **Contact details:**

21 Address: Division of Advanced Prosthetic Dentistry, Tohoku University Graduate
22 School of Dentistry, 4-1, Seiryomachi, Aoba-ku, Sendai, Miyagi, 9808575, JAPAN

23 Phone: +81-22-717-8369; Fax: +81-22-717-8371;

24 E-mail: nobuhiro.yoda.e2@tohoku.ac.jp

25 **Abstract**

26 Whilst the newly established biomechanical conditions following mandibular
27 reconstruction using fibula free flap can be a critical determinant for achieving
28 favorable bone union, little has been known about their association in a time-dependent
29 fashion. This study evaluated the bone healing/remodeling activity in reconstructed
30 mandible and its influence on jaw biomechanics using CT data, and further quantified
31 ~~its~~their correlation with mechanobiological responses through an *in-silico* approach. A
32 66-year-old male patient received mandibular reconstruction was studied. Post-
33 operative CT scans were taken at 0, 4, 16 and 28 months. Longitudinal change of bone
34 morphologies and mineral densities were measured at three bone union interfaces (two
35 between the fibula and mandibular bones and one between the osteotomized fibulas) to
36 investigate bone healing/remodeling events. Three-dimensional finite element models
37 were created to quantify mechanobiological responses in the bone at these different time
38 points. Bone mineral density increased rapidly along the bone interfaces over the first
39 four months. Cortical bridging formed at the osteotomized interface earlier than the
40 other two interfaces with larger shape discrepancy between fibula and mandibular bones.
41 Bone morphology significantly affected mechanobiological responses in the
42 osteotomized region ($R^2 > 0.77$). The anatomic position and shape discrepancy at bone
43 union affected the bone healing/remodeling process.

44

45 **Keywords:** Fibula free flap; Finite element analysis; Jaw biomechanics; Mandibular
46 reconstruction; Bone remodeling.

47

48 **1. Introduction**

49 Free vascularized osteocutaneous tissue transfer has become a well-established
50 procedure for maxillomandibular reconstruction following large resection due to trauma,
51 atrophy, and tumors ablation [1,2]. Fibula free flap (FFF) provides superior length and
52 long vascular pedicles for mandibular reconstruction, with proven subsequent high
53 reliability and adaptability [3]. Nevertheless, some clinical complications remain with
54 delayed or poor union between the grafted fibula bone and host native mandible [4,5].
55 Recent CT evaluations reported 20% [6] and 9% [7] non-union rates, respectively. Bone
56 union determines the strength and health of the reconstructed mandible, both of which
57 are essential for further ~~bone~~ occlusal and prosthetic rehabilitation. In the case of bone
58 fracture healing, the mechanobiological environment, which is thought to regulate
59 cellular behaviors, can be a critical determinant [8].

60 Unlike general bone fracture healing processes, FFF mandibular reconstruction
61 may be affected by additional factors, such as shape discrepancy between different
62 bones and poor bone vascularity [4,9]. Further, the loss of several masticatory muscles
63 due to resection can cause unbalanced jaw movement and abnormal mastication, leading
64 to significant change in the biomechanical conditions [10,11]. Thus the
65 mechanobiological responses in the jaw can be altered significantly; and such a change
66 in-turn affects subsequent bone remodeling activities [12,13]. To assist surgical planning
67 and oral rehabilitation it is essential to understand bone healing/remodeling activity and
68 its influence on jaw biomechanics, thereby preventing delayed or poor union of bone
69 grafts.

70 [Finite element \(FE\) analysis has the adequacy for the biomechanical studies on](#)

71 | orthopaedic [14-16] and dental problems [17-19]. Several those studies demonstrated
72 | their compelling advantages for understanding the biomechanics and mechanobiology
73 | of reconstructed mandibles *in-silico* [20,21]. ~~Several finite element (FE) studies~~
74 | ~~demonstrated their compelling advantages for understanding the biomechanics and~~
75 | ~~mechanobiology of reconstructed mandibles [14,15].~~ With recent advances in micro
76 | computerized tomography (CT), bone mineral density (BMD) and morphological
77 | changes can be measured to evaluate bone remodeling sequences noninvasively
78 | [~~16,17~~13,22,23]. The CT-based 3D FE models can be thus created to quantify
79 | biomechanical responses to functional forces in a patient-specific and time-dependent
80 | manner [~~13,18~~24,25].

81 | This study aims to (1) examine longitudinal changes in bone morphology and
82 | mineral density in the course of healing/remodeling after mandibular reconstruction
83 | with FFF; and (2) investigate the associated variation in mandibular biomechanics in
84 | terms of mechanical stimulus. The postoperative CT scans were performed at 4 critical
85 | time points over two and half years' clinical follow-up, and the CT images were
86 | segmented for both 2D multiple planar reconstructions (MPR) and 3D (volumetric)
87 | analyses. The bone condition was analyzed in both spatial and temporal manner, in
88 | terms of morphology and BMD. Nonlinear 3D FE analyses were conducted to quantify
89 | the bone mechanobiological stimuli at the se different time points; and then correlated to
90 | the corresponding *in-vivo* clinical data. By establishing this combined *in-vivo* and *in-*
91 | *silico* approach, the mutual influence between tissue conditions and mandibular
92 | mechanobiology was assessed. The results are expected to provide important insights
93 | into surgical plan for mandibular reconstruction.

94 **2. Materials and Methods**

95 ***2.1 Clinical Treatment***

96 A 66-year-old male patient received mandibular reconstruction with
97 osteotomized FFF, due to a squamous-cell carcinoma at the right molar gingiva at the
98 Department of Otolaryngology-Head and Neck Surgery, Tohoku University Hospital in
99 Japan. Upon harvesting, the fibular bone was segmented to match the defect jaw
100 morphology. A titanium fixation plate (Synthes, Solothurn, Switzerland), which was
101 pre-bent using the CT-based 3D patient model before surgery, was configured to be
102 fixed monocortically with a total of 11 titanium screws (Synthes, Solothurn,
103 Switzerland) as shown in Fig. 1. The first CT scan (M0) was performed at the end of
104 surgery, and the follow-up CTs were taken at 4, 16 and 28 months after surgery (namely,
105 M4, M16, and M28, respectively). A removable partial denture was inserted into this
106 subject 6 months after the surgery; however, the subject did not use it for mastication,
107 due to fear of biting on the reconstructed side. The periodontal conditions of the
108 remaining teeth and the removable partial denture have been maintained at the
109 Maxillofacial Prosthetics Clinic in Tohoku University Hospital every three months.

110 ***2.2 CT Imaging Acquisition and 2D Image Analysis***

111 Multi-detector helical CT scans were performed for the follow-up examinations
112 using Somatom Emotion 6 (Siemens, Erlangen, Germany) at 120 kV and 80 mA with
113 the spatial resolution of 0.4, 0.4, and 0.8 mm in the radial, tangential, and axial
114 directions. The CT data was further processed with the medical image viewer software
115 (EV Insite S, PSP Co., Tokyo, Japan), for the detection and alignment of anatomic
116 landmarks between the different cross-sectional examinations. The mandibular plane

117 was defined using three reference points; namely, left Gonion point, Menton point, and
118 inflection point of a titanium fixation plate (green triangles in Fig. 2a). Six planes
119 parallel to this mandibular plane were selected for the quantitative analysis of bone
120 union at three docking sites (DS1, DS2, and DS3, respectively) with 2 mm intervals by
121 multiple planar reconstructions (MPR) (Fig. 2b) [12]. On each plane, a 2 mm³ volume
122 of interest (VOI) was ~~placed~~considered along the superior-inferior axis (Fig. 2b). Since
123 a significant correlation between Hounsfield units (HU) obtained from clinical CT scans
124 and bone mineral density (BMD) were ~~found~~established [1926], the HU values change
125 in VOIs can be regarded as the BMD changes over time here, particularly for bone
126 unification at the contact interfaces. All the VOIs were placed at the same positions
127 throughout these four time points, based on the distance from the titanium fixation plate
128 and screws as a reference.

129 ***2.3 3D Registration and Volumetric Analysis***

130 3D registration was carried out for investigating the longitudinal changes in
131 bone surface profile and mineral density using Amira 2016.22 (Zuse Institute Berlin
132 (ZIB), Berlin, Germany) (Fig. 3a). The titanium fixation plate was selected as the
133 reference geometry for its rigidity and high contrast. To quantify the variation of BMD
134 at the docking sites, the change in greyscales was correlated with the distance from the
135 inferior to the superior aspect. The average value of the pixel intensity (i.e. greyscale)
136 was calculated in the cortical bone region on each slice (at a regular spacing of 0.8 mm
137 along the coronal ~~axis~~axial), enabling a plot of pixel value change along the axial direction.
138 To determine the HU values of the cortical bone, several profile lines were constructed
139 at the CT images cross the region of mature cortical bone. By sampling the histogram
140 distribution, a HU value of 1536 was determined to be a threshold for determining

141 | cortical bone pixels, which is ~~in~~-consistent with the reported HU value of cortical bone
142 | for cone beam CT in literature [2027]. By using this cortical bone threshold, variation in
143 | both bone density and volume at the same region for the four time points were
144 | quantified. The detailed variation in bone volume (i.e. volume of the cortical bone voxel
145 | cuboids) along this direction was plotted using the same approach. In addition, the
146 | variation in pixel number, rather than the pixel intensity, in the cortical bone region was
147 | considered.

148 | ***2.4 Finite Element Analysis***

149 | Four case-specific FE models were created based on the CT data taken at M0,
150 | M4, M16, and M28, respectively [21,22,28,29]. The CT images were imported into
151 | ScanIP Ver. 4.3 (Simpleware Ltd, Exeter, UK) for segmentation. The segmented masks
152 | (bone, individual tooth and titanium fixation plate) were further processed in
153 | Rhinoceros 4.0 (Robert McNeel & Associates, Seattle, USA) to create parametric
154 | models with non-uniform rational B-spline (NURBS) (Fig. 3b). Following the
155 | development of the mandibular models, the total 11 fixation screws were modeled
156 | according to the manufacturing specifications in Solidworks 2013 (SolidWorks Corp,
157 | Waltham, MA, USA). Those screws were virtually inserted into the models in
158 | Rhinoceros 4.0 as guided by the CT images. Considering that the patient disuse the
159 | denture in his daily life and has no parafunctional habit, the denture was not inserted in
160 | the models. To ensure the numerical accuracy, an adaptive mesh was generated based on
161 | a mesh convergence test. Ten-node Qquadratic tetrahedral elements with hybrid
162 | formulation (C3D10H) were adopted to ensure smoothness along the contact interfaces.

163 | A pixel-based mapping algorithm was adopted to create the heterogeneous bone
164 | density distributions at the different time points, reflecting the changes of the

165 | anatomical conditions [29].²² A homogeneous isotropic linear-elastic model was used to
166 | define the teeth (Young's modulus $E = 20,000$ MPa, Poisson's ratio $\nu = 0.2$), titanium
167 | fixation plates and screws (Ti6Al7Nb: $E = 110,000$ MPa, $\nu = 0.3$) [15,23,21,30].

168 | The hinge constraints were prescribed for the corresponding mandibular
169 | condyles. In this subject, the large bone resection was accompanied by the functional
170 | loss of the right masseter, medial pterygoid and temporalis muscles; and consequently
171 | masticatory conditions changed dramatically post-surgery. Due to lack of information
172 | regarding muscular forces after such a large resection [420], the magnitudes and
173 | directions of individual forces were derived based on the literature for the remaining
174 | muscles (masseter muscle: 59.23 N, medial pterygoid muscle: 39.60 N, lateral pterygoid
175 | muscle: 34.44 N, and temporalis: 34.09 N, respectively) [2431].

176 | Strain energy density (SED) was quantified as a mechanobiological stimulus to
177 | analyze the bone responses in the three docking sites and VOIs. SED has been
178 | considered an effective stimulus to bone remodeling in long bones [32] and mandible
179 | [24,33] and can be a scalar quantity to combine stress and strain but eliminate their
180 | directionalities [34]. The SEDs at different time points were correlated with the
181 | corresponding change in the bone density. In this study, linear regression analysis was
182 | performed using IBM SPSS Statistics Ver. 21.0 (IBM Corp., New York, NY, USA) to
183 | examine the correlations between stimuli and bone remodeling progression in all VOIs.
184 | The R^2 values presented the goodness of fit for the predictor functions, thereby
185 | indicating the extent of correlation.

186 3. Results

187 3.1 MPR Image Assessment for Bone Morphology and Mineral Density

188 Fig. 4 shows the longitudinal changes in bone profile from the CT-based MPR
189 images. In docking site DS1, a significant amount of callus bone formed at time point
190 M4, and the cortical bridging successfully formed in both buccal and lingual regions at
191 M16. In DS2, the cortical bridging formed at M4 in both the buccal and lingual regions.
192 Also, the cortical-like bone appeared to fill the entire interface, while some resorption
193 occurred at the upper and bottom surfaces of cortical bone. In DS3, there was large
194 discrepancy of bone shape at the initial stage. However, the bone shapes gradually
195 remodeled and cortical bridging was found in both the buccal and lingual regions at
196 M16.

197 ~~Fig. 5 shows that the averaged HU value was calculated for each VOI to~~
198 ~~quantify the change of BMD. Averaged HU value was calculated for each VOI to~~
199 ~~quantify the change of BMD (charts in Fig. 4).~~ For DS1, both superior and inferior
200 cortical bones underwent resorption from M0 to M16, while the BMD peaked in the
201 trabecular interface regions at M4 before undergoing resorption. In contrast, the grafted
202 bones at DS2 performed exceedingly well in terms of new bone formation, despite
203 being osteotomized, ~~with-seen in~~ rapid increases of BMD in the first four months. For
204 DS3, the cancellous/trabecular region underwent much more ~~severe-dramatic~~
205 remodeling than the cortical bone with rapid increase in BMD from M0 to M4 but
206 decrease from M4 to M16.

207 **3.2 Volumetric Assessment of Bone Mineral Density and Morphology**

208 Bone morphological changes were visualized as the apposition and resorption on
209 | the bone surface by 3D volumetric registration in the three docking sites (Fig. ~~5a-e~~6).
210 | The longitudinal changes in bone volume were site-specific and the rate of volume
211 | increase in the cortical bone region was positive in all the three sites from M4 to M16
212 | (Fig. ~~5d7a~~). Fig. ~~5e-7b~~ exhibits the longitudinal change rate of bone volume at each
213 | docking site. Bone volume increased remarkably from M4 to M16 due to new bone
214 | formation, especially at the region from 15 mm to 25 mm for DS1 and from 20 mm to
215 | 30 mm for DS3 on the sectional plane of mandible as visualized in Fig. ~~5a-e~~6. Fig. ~~5f-7c~~
216 | plotted the site-specific change rate of BMD based on the average grayscale in the
217 | cortical bone region. Note that the BMD decreased in the first four months for all the
218 | docking sites.

219 **3.3 Mechanobiological Stimulus Distribution**

220 | Fig. ~~6a-8~~ shows the longitudinal changes in the SED distribution and
221 | corresponding CT MPR images of the reconstructed mandible. Both global and local
222 | SED distributions changed ~~significantly~~ with time significantly. The longitudinal
223 | changes in morphology and BMD were remarkable particularly for DS1, leading to
224 | substantial variation in the SED distribution.

225 The SED at VOIs in the cortical bone region was generally higher than that in
226 | the cancellous region in DS1 and DS2 (Fig. ~~6b9~~). At each VOI, the SED decreased with
227 | time at DS1 and DS3, especially in the superior region of DS1. While the increase in
228 | SED with time could be found in some VOIs, the SED dropped from M0 to M4 and
229 | then gradually increased till M28 (but never exceeds that at M0), at 6, 8, and 10 mm

230 VOIs in DS2.

231 Linear regression analysis between the HU values and SED in VOIs indicated
232 that there was a strong dependence on the HU values only in DS2 (p<0.05), as shown in
233 Fig. 10.

234 **4. Discussion**

235 Both 2D MPR images and 3D volumetric analyses enabled to quantify and
236 visualize time-dependent bone apposition and resorption in terms of morphology and
237 BMD in this FFF reconstructive mandible. This study is believed to be the first of its
238 kind for investigating the anatomical sequence of healing/remodeling process and its
239 correlation with mechanobiological responses in a reconstructive mandible.

240 The clinical process of cortical bridging at bone docking regions was found to be
241 significantly site-specific based on the results of both 2D MPR images and 3D
242 volumetric analyses. Biological healing at bone union is influenced by complex cellular
243 and molecular activities, and can be affected by the dimension of bone segment gap
244 [2535] and contact shape [9]. In this study, we set up a criterion to justify the cortical
245 bridging, namely, no gap was observed between the two bones in the six cross sectional
246 planes as shown in Fig. 4. According to this criterion, the contact region in DS2
247 achieved earlier cortical bridging than the other two sites. Specifically, the contact region
248 in DS2 achieved earlier cortical bridging than the other two sites (Fig. 4).

249 The BMD became higher within the first four months in all the VOIs except for
250 the cortical bone regions in DS1 (Fig. 45). Those cortical regions appeared to undergo
251 significant resorption, while the osseous callus was generally found at the interface of

252 | trabecular regions during the bone-healing phase [26,2736,37]. The BMDs of all the
253 | cortical bone regions in the docking sites were also found to decrease in the first four
254 | months, which was most remarkable for DS2 (Fig. 547c). Despite a vascularized bone
255 | graft, the lower bone vascularity may have caused the reduction of BMD on the cortical
256 | region of the fibula graft [28,2938,39]. Despite the lowered BMD, 3D volumetric
257 | analysis revealed a higher increase rate of bone volume in DS1 than the other two sites
258 | over the same time period (Fig. 547a). Primary bone apposition may have developed
259 | throughout formation of the osseous callus at the endochondral and periosteal areas
260 | (Figs. 4a4 and 5) [2535]. The woven bone with low BMD appears to initially form for
261 | filling the gap and reducing morphological discrepancy, which may be related to the
262 | initial volume increase in DS1. Lamellae bone with high BMD appears to form after M4
263 | [2737]. Lower bone vascularity in the distal segment of osteotomy [2939] may limit
264 | those biological healing activities in DS2 and DS3 compared to DS1, further
265 | contributing to the initial reduction in the bone volume (Fig. 547a).

266 | Considering the positive increase rates attributable to bone apposition at all three
267 | docking sites from M4 to M16, bone (re)modeling activity had a primary effect on post-
268 | healing bone formation [30,3140,41]. Osseous callus at the interface regions in DS1 and
269 | DS3 gradually became cancellous bone, forming a natural mandibular structure during
270 | the course. Nevertheless, the healing and remodeling process at the docking site,
271 | especially with large shape discrepancy, is considered to be significantly slower than
272 | those of the general bone fracture [27,3237,42]. Note that the mandible can be distorted
273 | during daily oral function [3343]. Despite the mechanical fixation by titanium plate, the
274 | distortion can affect the mechanical stability of the docking sites, which might also
275 | delay the healing process [9].

276 Mechanical loading is known to stimulate bone healing and remodeling process,
277 likely enhancing bone mass and functionality [3040]. The mechanobiological impetus
278 can thus be related to the bone remodeling activity [12,13]. SED has been considered an
279 effective stimulus to bone remodeling in long bones [3432] ~~and as well as~~ mandible
280 [18,3524,33]. This study revealed the correlation between SED and healing/remodeling
281 outcome over the time period concerned.

282 The variation in SED distribution was attributed to the time longitudinal change
283 in the mandibular morphology (Figs. 68 and 9), as well as load transfer in the
284 restructured mandible, particularly through the fibula grafts. In other words, the
285 functional load was initially transferred to the fibula graft completely via the titanium
286 fixation plate (M0); but subsequently, a greater proportion of load transferred through
287 the bony tissue as the extent of bone union increased. In addition, the remaining
288 unbalanced muscle activities readapt with time [10,11]. All these factors have a
289 collective effect on the mechanobiological responses.

290 As shown in Fig. 710, the SED had a strong dependence on the HU values in
291 DS2 ($p < 0.05$). The HU value altered the load bearing capability of the fibula bone,
292 meaning that the SED is associated with HU values. Lower bone vascularity and good
293 bone contact condition at DS2 possibly enhance the effect of mechanobiological stimuli
294 on BMD adaptation, which might be related to the earlier process of cortical bridging at
295 the DS2. For DS1 and DS3, significant shape discrepancy due to reconstruction
296 generated non-physiological stress/strain concentration, which might have distorted the
297 distribution of SED and its correlation to remodeling.

298 Clinically, the implant-supported denture is considered as the most suitable

309 option for functional rehabilitation following mandibular reconstruction [2]. Although
300 the timing of implant placement is still controversial, several studies adopted the time
301 for implant placement at least 6–12 months after the reconstruction with FFF
302 [1,36,37,44,45]. Considering the cortical bridging as a predictor of bone union strength
303 [7,38,46], all the bone unions can be confirmed through CT scanning, especially in the
304 cases with a large bone discrepancy. Specifically, favorable initial bone contacts with
305 small shape discrepancy are considered a primary factor for earlier success of cortical
306 bridging.

307 There are still some limitations in this study. Constrained by the clinical protocol
308 and radiation dosage allowance, the scanning resolution of CTs could have affected
309 modeling accuracy. The FE analyses still included several assumptions, such as
310 simulation under static loading conditions and rotational movement on the mandibular
311 condyles. The applied muscle forces did not precisely reflect specific condition of this
312 subject; plus the muscle forces are anticipated to change over time after reconstruction
313 [39,40,47,48]. Consequently, the resultant reaction responses on both
314 temporomandibular joints might become asymmetric and physiologically complicated.
315 Finally, while the study was featured as patient-specific, the results were based on only
316 one particular subject. In addition, other patient's factors, such as the systematic
317 background and the treatment process, could be generally the decisive factors to the
318 bone healing and remodeling process at the docking sites. Further evaluation and data
319 acquisition of other subjects with inevitably varied conditions are necessary before
320 generalizing these clinical and biomechanical findings.

321 **5. Conclusion**

322 | This newly developed ~~analysing methods~~analysis procedure provided a
323 | quantitative clinical follow-up of mandibular reconstruction with fibula free flap (FFF)
324 | and fundamental understanding of time-dependent biomechanical responses in the
325 | reconstructed mandible. It was found that the bone healing and remodeling process at
326 | the docking sites were site-specific; and cortical bridging in the osteotomized region
327 | took place faster than that in the other docking sites between mandibular and fibula
328 | bones for the specific patient concerned. Within the limitation of this study, the
329 | anatomic position and the discrepancy of initial shape at the docking sites between the
330 | host mandible and fibula graft affected the bone healing and remodeling process. It
331 | ~~revealed~~divulged a correlation between mechanobiological stimulus (strain energy
332 | density - SED) and the longitudinal change in bone mineral density (BMD) and
333 | morphology, especially at the osteotomized region. The longitudinal CT data and
334 | mechanobiological correlation generated in this study provided new insights into
335 | patient-specific surgical planning and occlusal rehabilitation.

336

337 **Conflict of interests**

338 None declared.

339

340 **Ethical approval**

341 The research protocol was approved by the research ethics committee of the Tohoku
342 University Graduate School of Dentistry (reference #24-10). Full written informed
343 consent was obtained to use the CT images for this study. All procedures performed in
344 this study were in accordance with the ethical standards of the 1964 Helsinki
345 Declaration (<http://www.wma.net>) and its later amendments.

346

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352

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512

1 Captions to illustrations

2 **Figure 1. Intraoperative view illustrating the fibula bone affixed to the titanium**
3 **fixation plate.**

4 White triangle: mandibular bone, Black triangle: fibula bone. **Green arrows: Screw**
5 **position (8 of 11 screws are shown in this picture).** The flap pedicles were anastomosed
6 with the thyroid artery and the external jugular vein.

7

8 **Figure 2. Clinical X-ray and CT images for assessment.**

9 (a) Postoperative radiograph (M0). Yellow boxes: three investigated docking sites (DS1,
10 DS2 and DS3) for the bone union. Green triangles: reference points for defining
11 mandibular plane for 2D MPR (multiple planar reconstructions) analysis. (b) CT MPR
12 cross-sectional images of contact interface perpendicular to the mandibular plane (green
13 line in (b)) at three docking sites at M0; brown: mandible, yellow: anterior fragment of
14 fibula bone, green: posterior fragment of fibula bone. Lateral lines: planes for analysis,
15 boxes: cubic (**2 mm³**) volume of interests (VOIs).

16

17 **Figure 3. Procedure of 3D image registration and computational model for finite**
18 **element analysis.**

19 (a) Procedure of 3D image registration for investigating the longitudinal changes in
20 bone surface profile and mineral density; the example for the DS1 between M0 **model**
21 **(orange)** and M4 **model (blue)**. Titanium fixation plate was selected as the reference
22 geometry for the registration. (b) 3D modeling for the patient's jaw model (M0) with

23 non-uniform rational B-spline (NURBS).

24

25 **Figure 4. MPR CT image analysis.**

26 (a) DS 1, (b) DS 2, (c) DS 3. Individual planes and VOIs are defined in **Figure 2**. Each
27 **plane** position stated in terms of the distance from the bottom. Both top and bottom
28 planes included the cortical bone region of fibula graft at M0.

29

30 **Figure 5. Time-dependent changes in HU value.**

31 (a) DS 1, (b) DS 2, (c) DS 3.

32

33 **Figure 6. Volumetric analysis of bone morphology changes by 3D image**
34 **registration and superimposition.**

35 (a) DS 1, (b) DS 2, (c) DS 3

36

37 **Figure 7. Volumetric analysis of bone morphological changes.**

38 (a) Volume increase rate in the cortical bone region, (b) Site-specific volume change
39 rate (%), (c) Site-specific BMD (greyscale) increase rate (%) based on the grayscale on
40 the cortical bone region.

41

42 **Figure 8. Mechanobiological stimulus distributions.**

43 (a) M0, (b) M4, (c) M16, (d) M28. SED distribution was shown at the different time

44 points and in different regions along with corresponding CT MPR images (anterior end
45 of fibula graft in DS1 and posterior end of fibula graft in DS3).

46

47 **Figure 9. Average values of SED in each VOI assigned in the same location as in**
48 **the CT MPR image.**

49 VOI position stated in terms of the distance from the bottom at each docking site shown
50 in Fig. 2.

51

52 **Figure 10. Linear regression analysis between CT Hounsfield Unit (HU) and SED**
53 **in volume of interests (VOIs)**

54 The VOIs were on the same location in each multiple planar reconstruction (MPR)
55 image at each docking site shown in Fig. 2. *P < 0.05, **P < 0.01.

Figure 1
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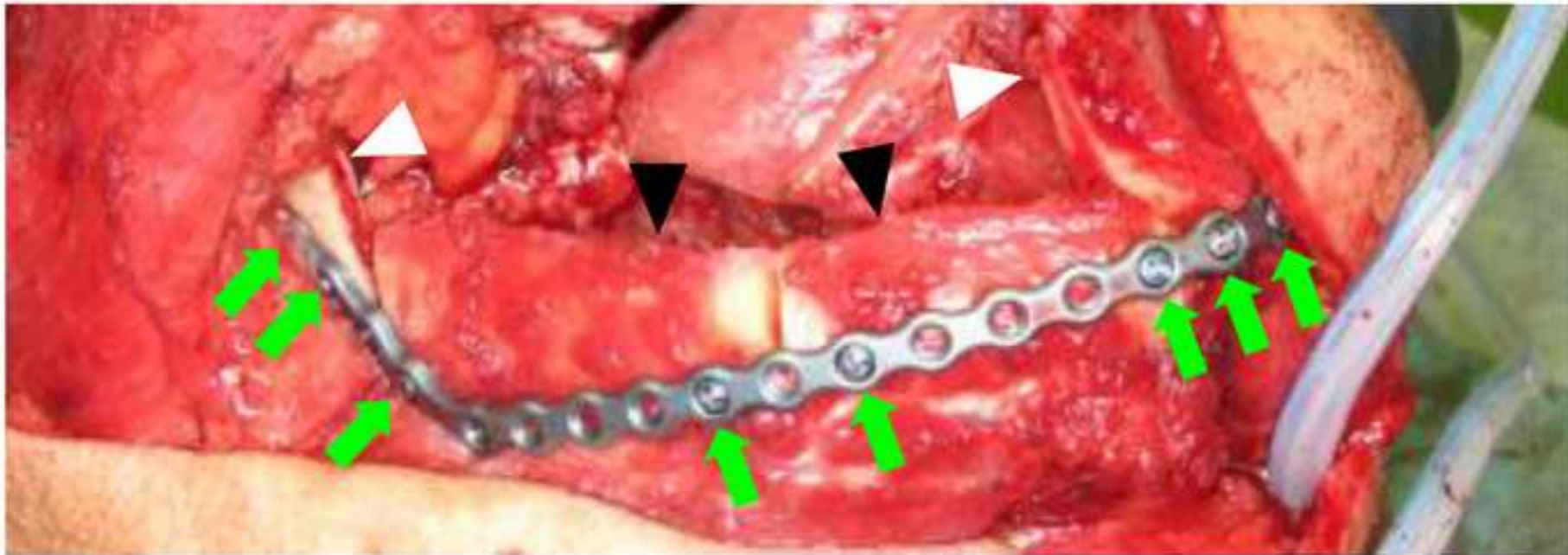
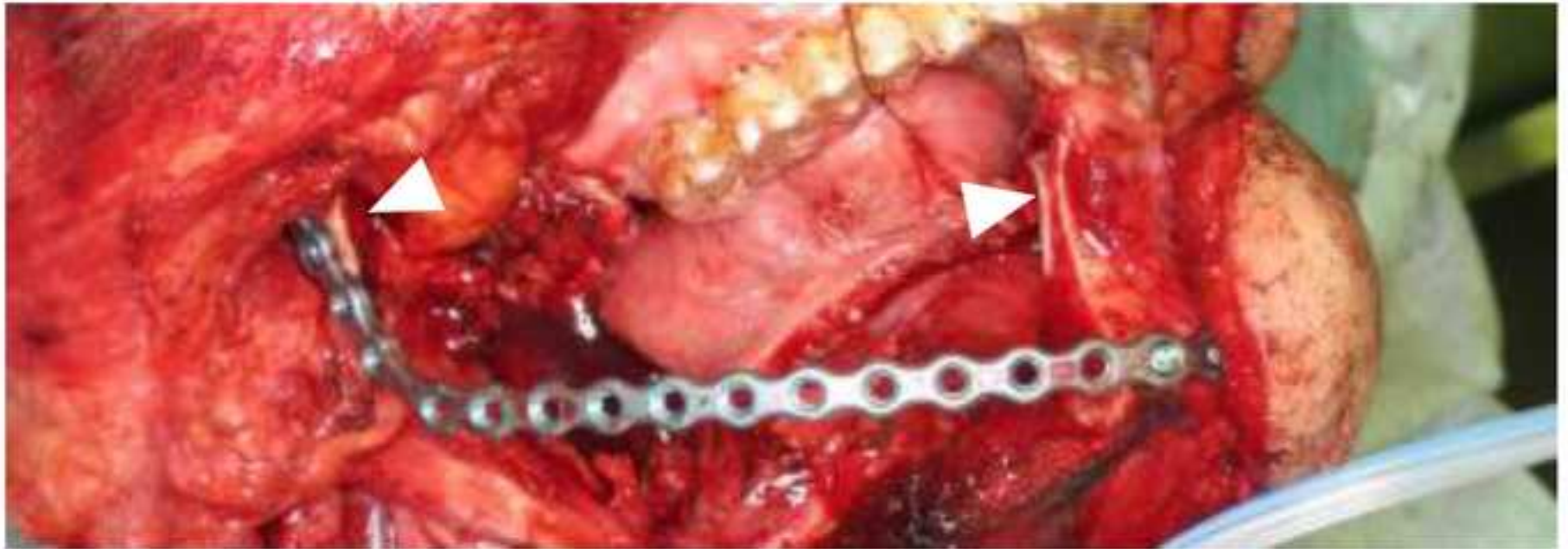


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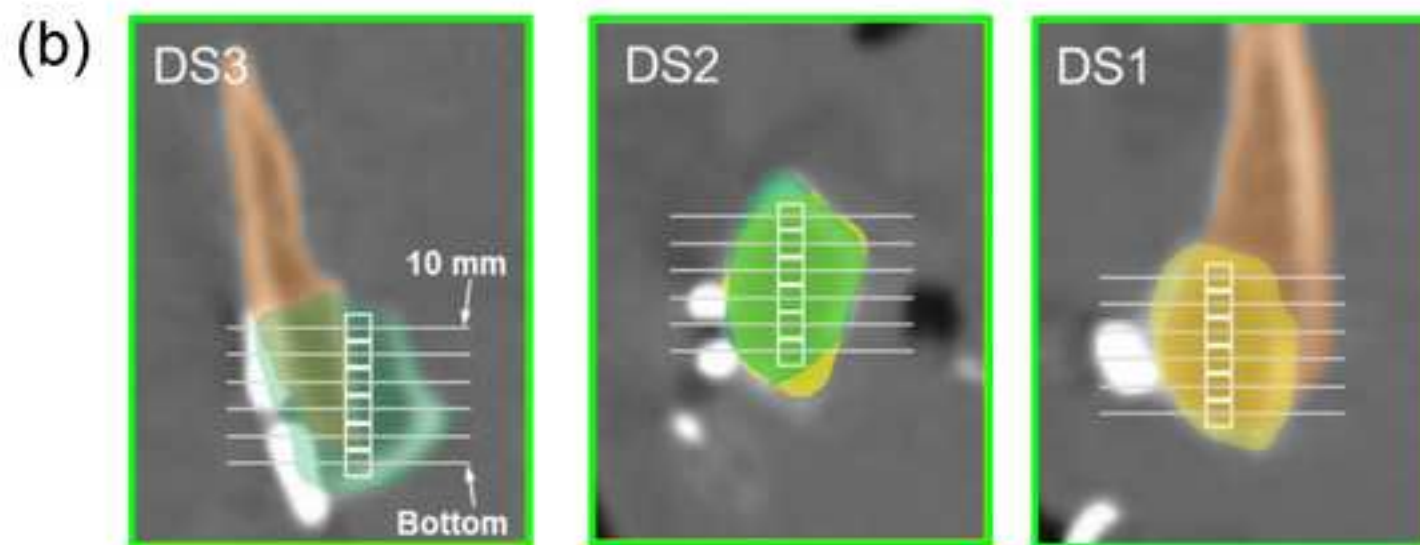
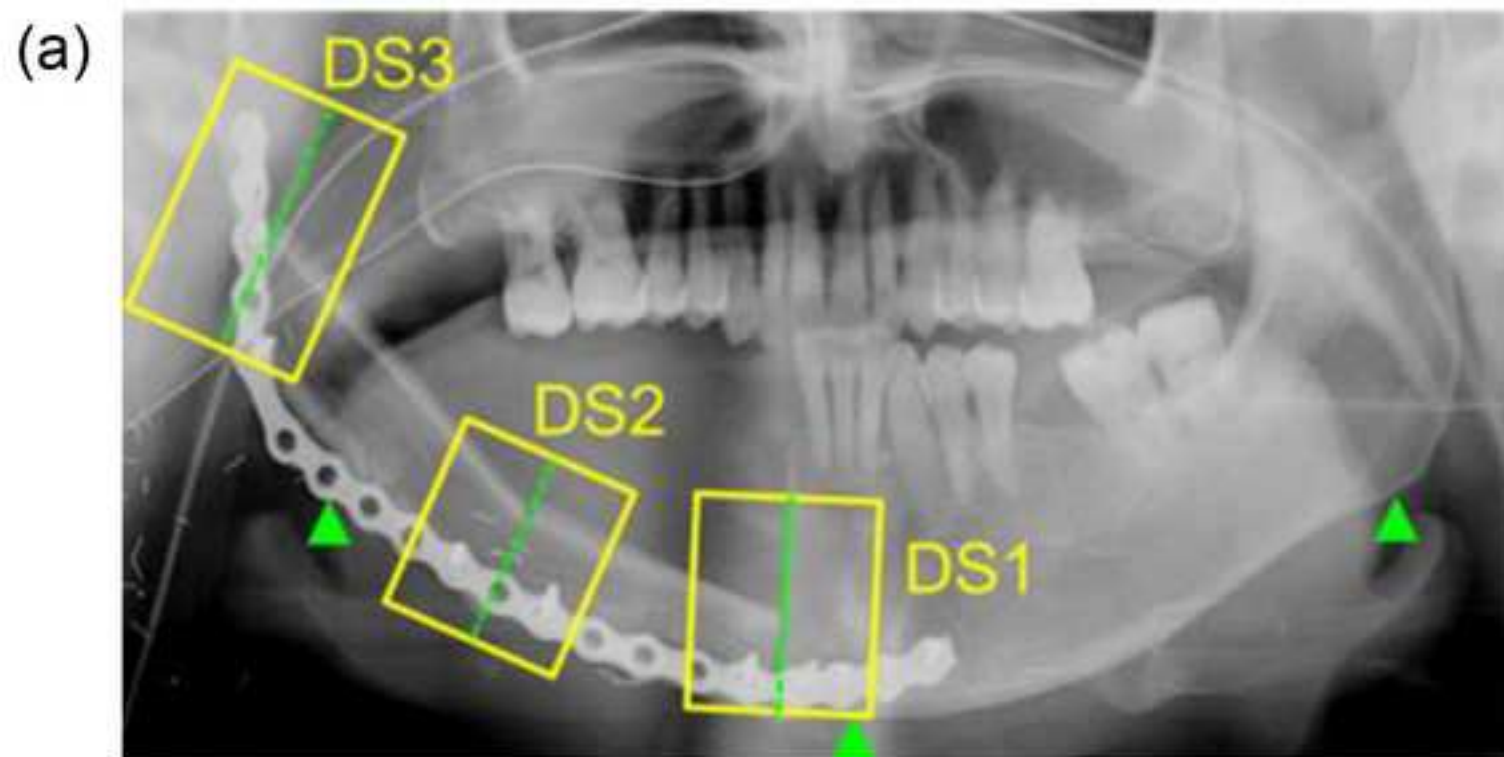


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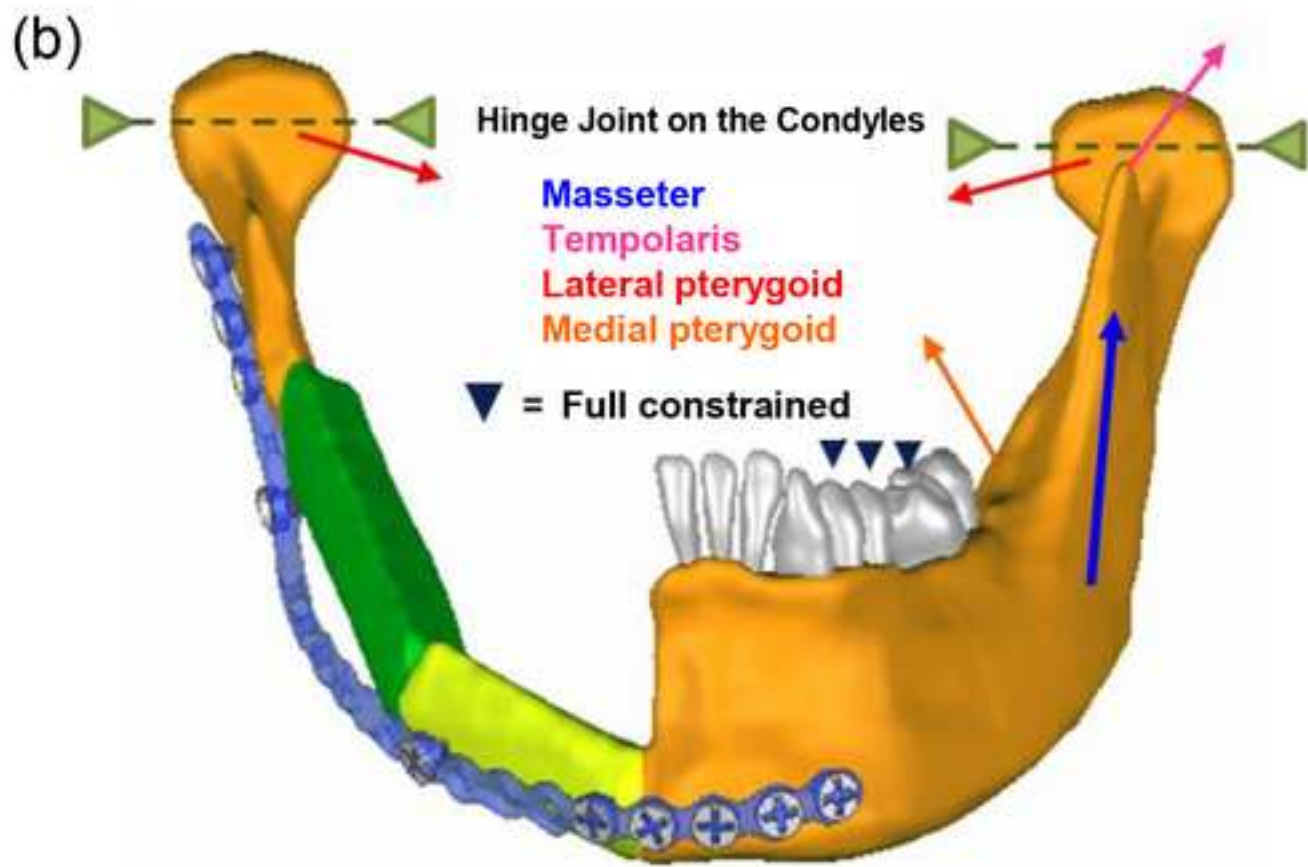
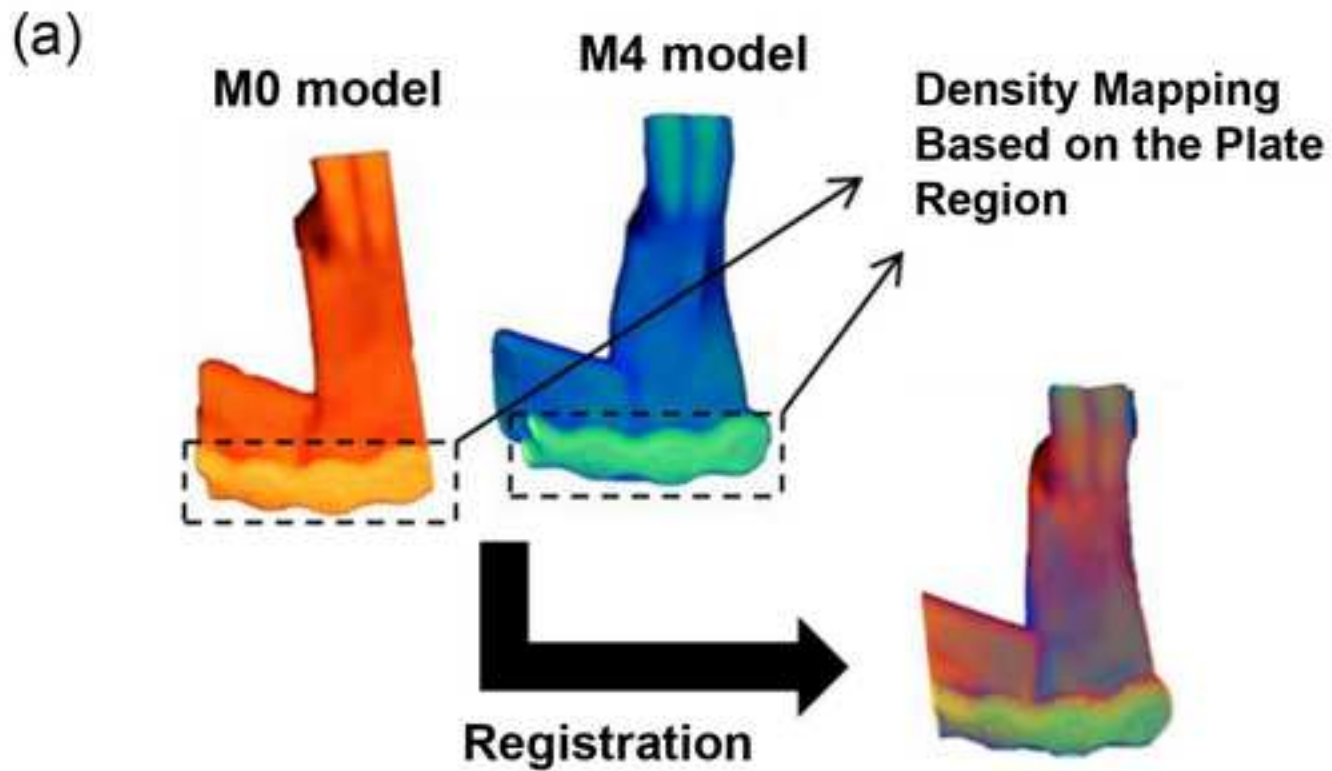


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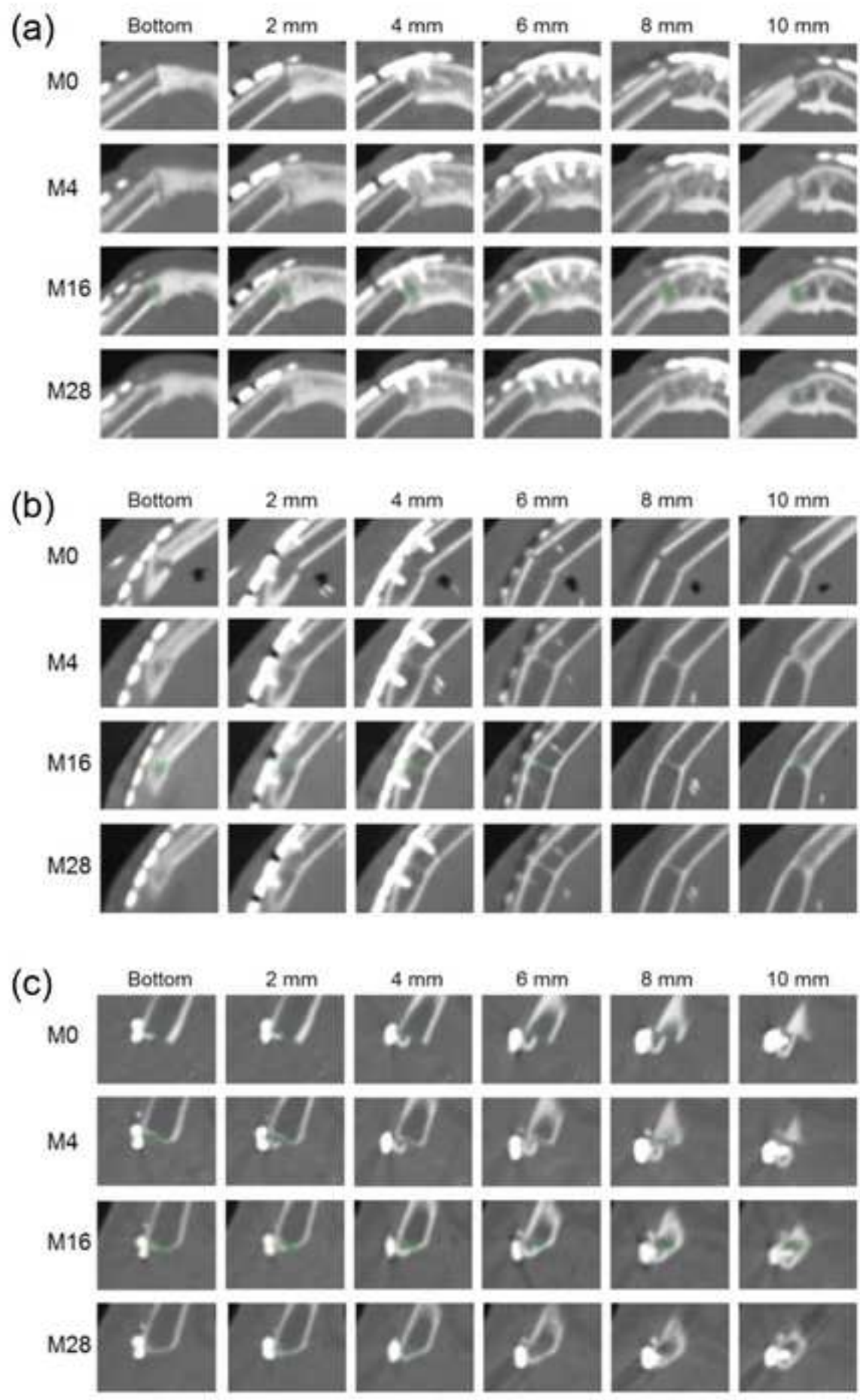


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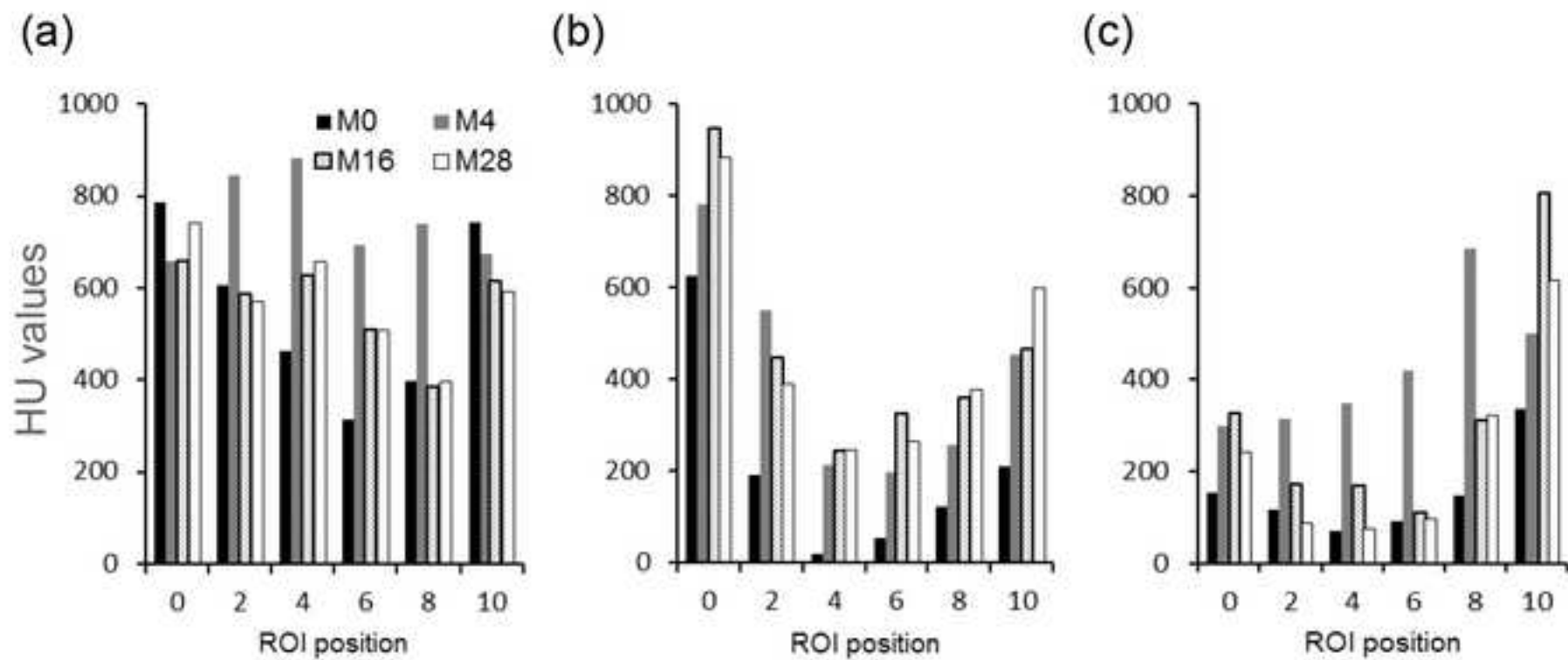


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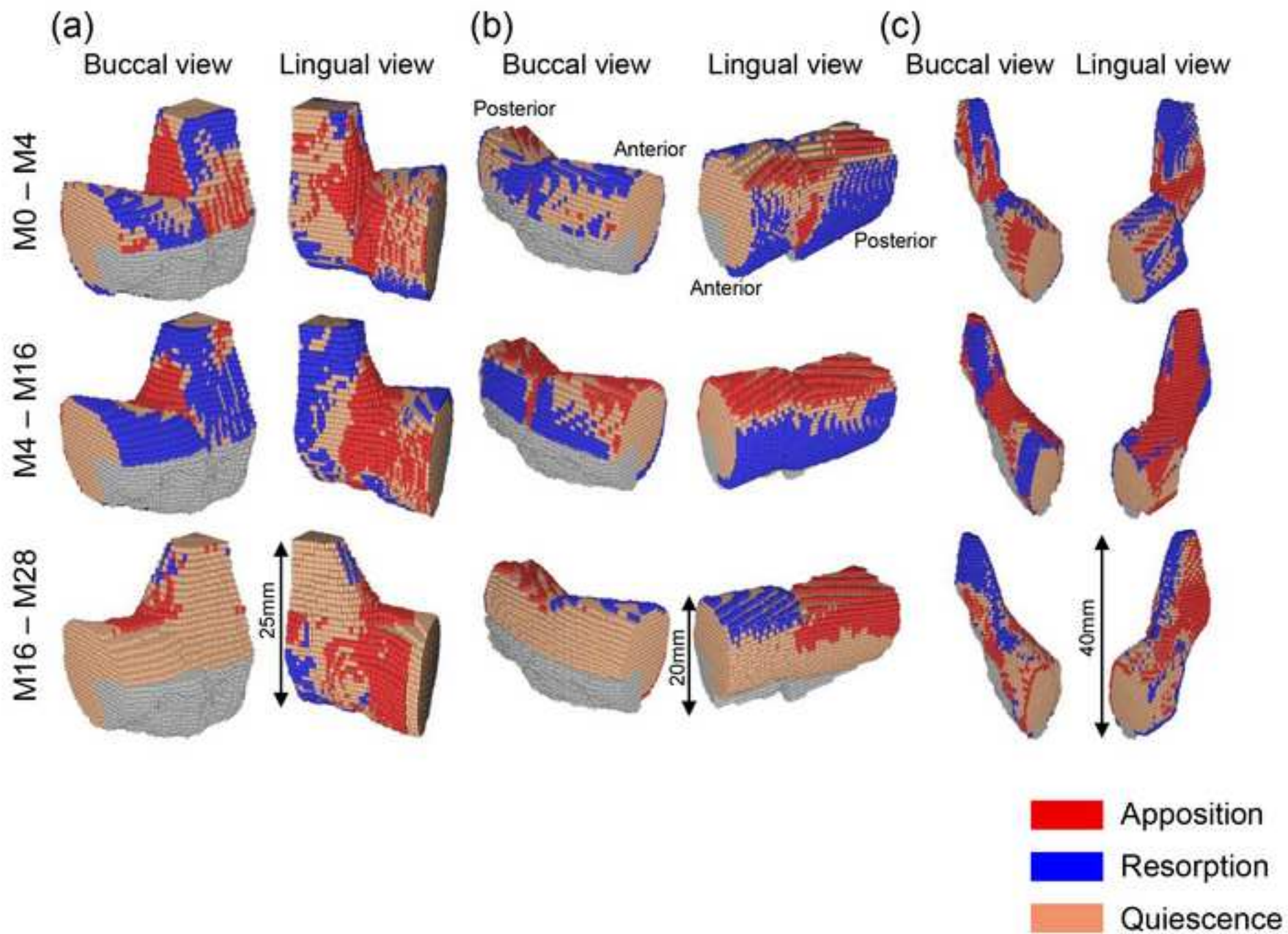


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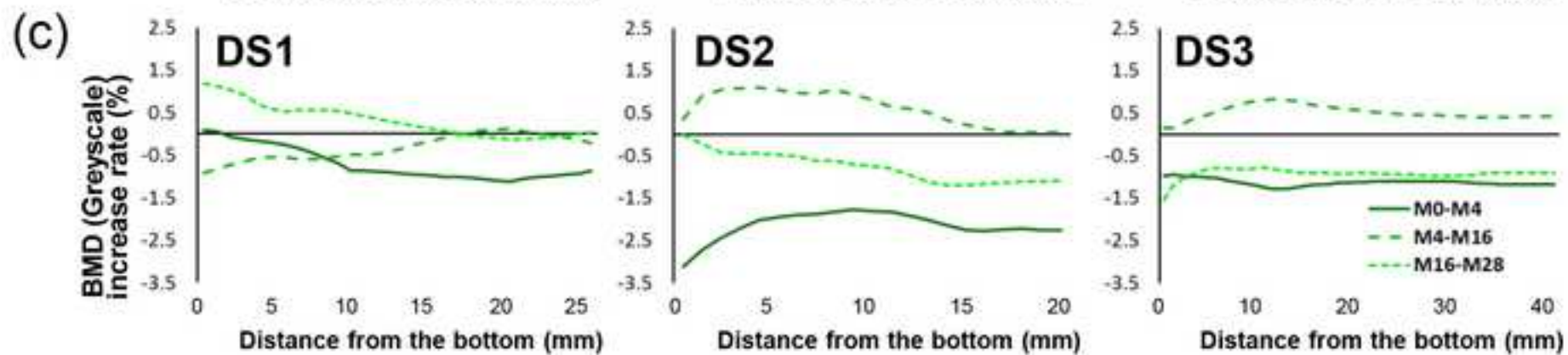
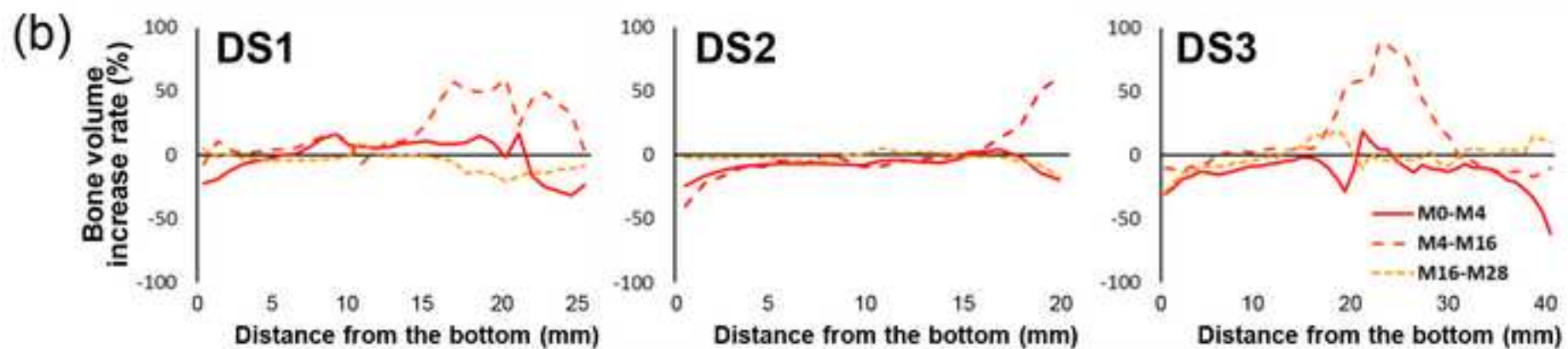
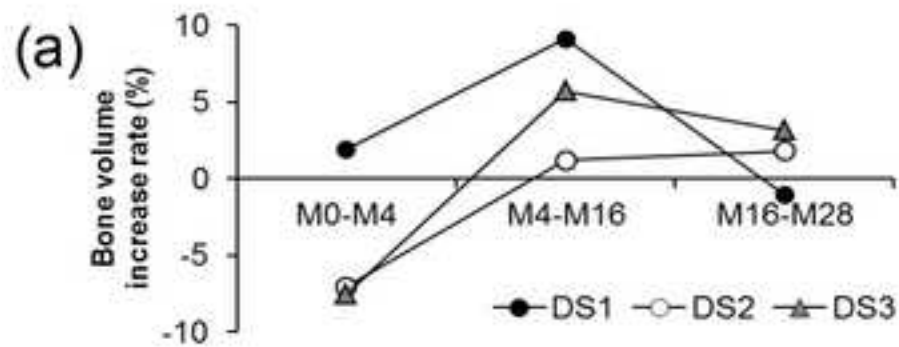


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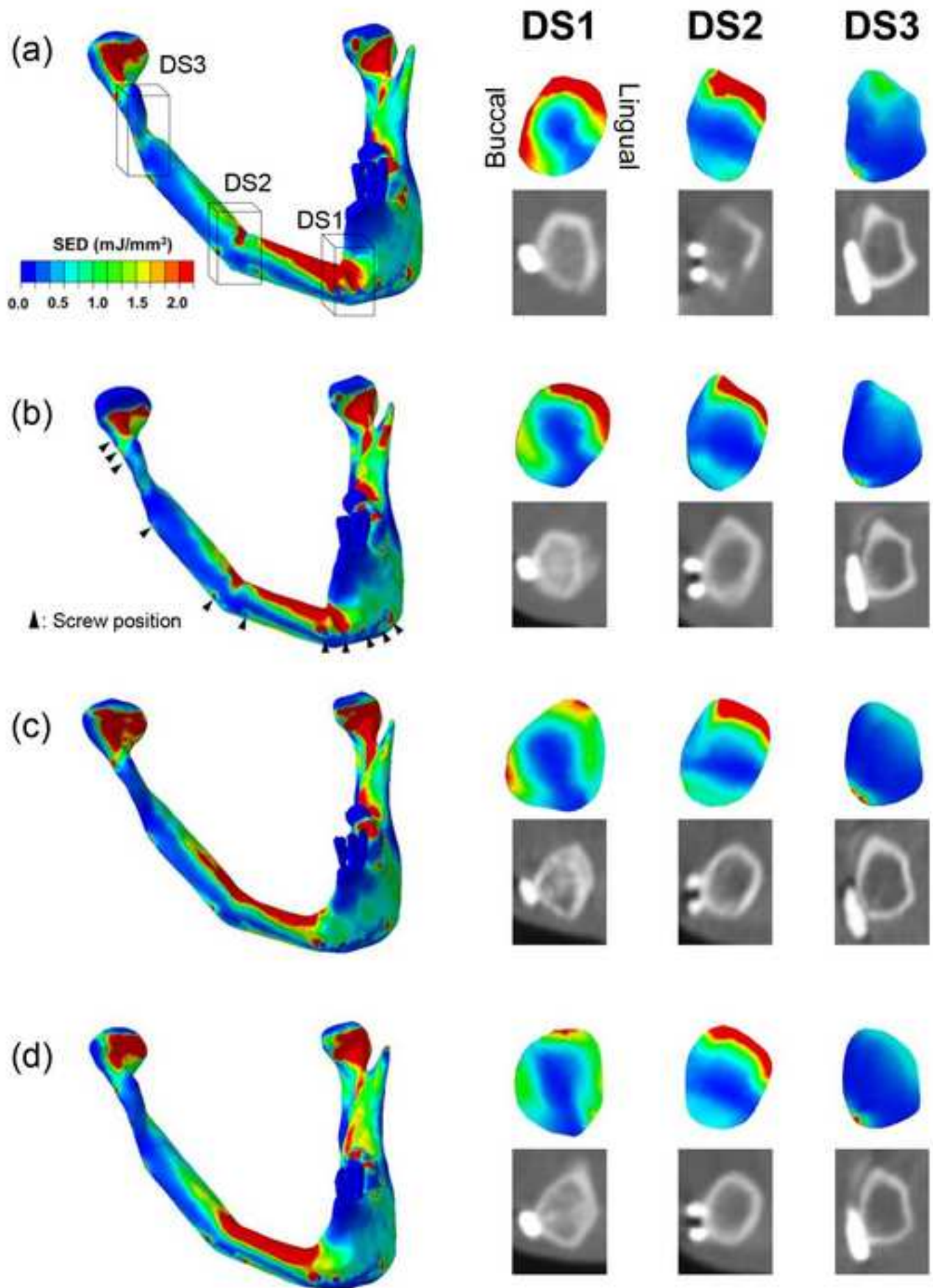


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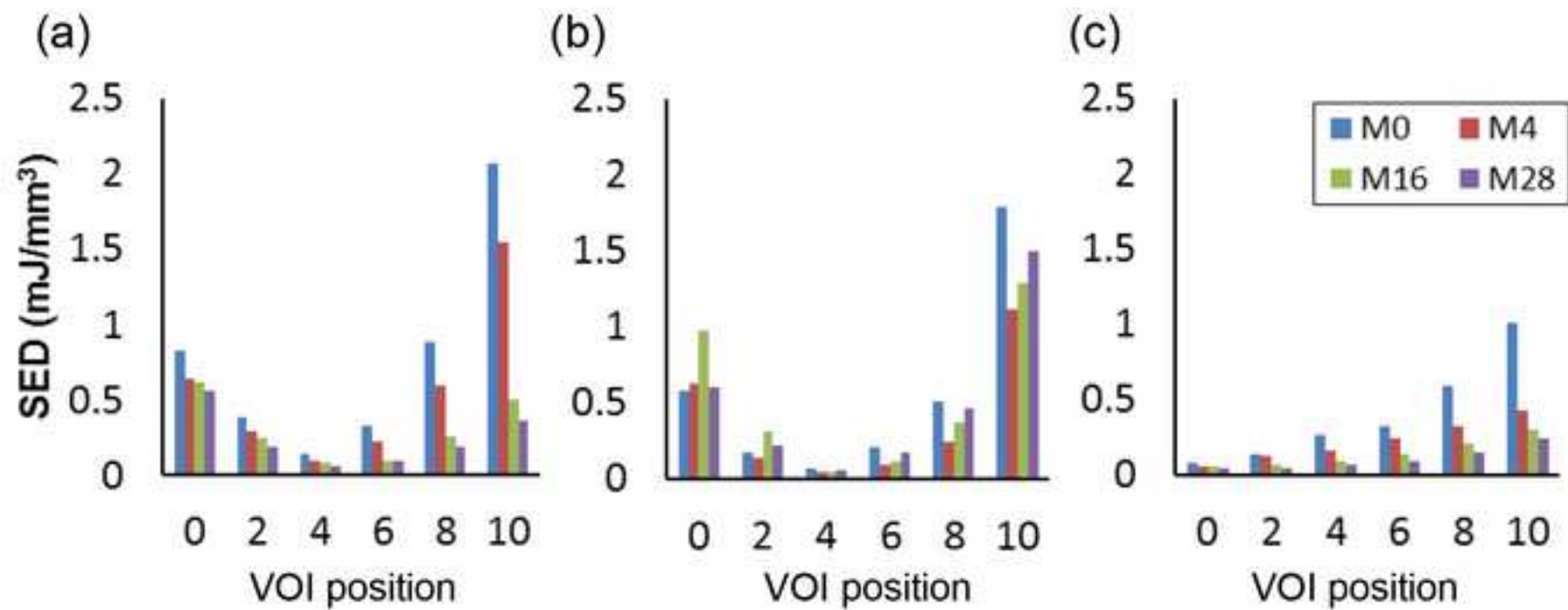


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