

1 **Title:** Enhanced bone healing using collagen-hydroxyapatite scaffold
2 implantation in the treatment of a large multiloculated mandibular
3 aneurysmal bone cyst in a Thoroughbred filly

4

5 **Running Head:** Collagen-HA scaffold shows enhanced bone healing
6 in a clinical case study

7

8 **Authors:**

9 * Florent David^{1,2}, DVM, MSc, Dipl. ACVS/ECVS, ECVDI Assoc., Dipl.
10 ACVSMR

11 * Tanya J. Levingstone^{3,4,5}, MSc, BEng, PhD

12 Wilfried Schneeweiss^{1,6}, MVM, Dr. med. Vet.

13 Marie de Swarte¹, DVM

14 Hanne Jahns¹, MVM, PhD, Dipl. ECVP

15 John Gleeson^{3,4,5,7}, BA, BAI, MSc, PhD, MIEI

16 Fergal J. O'Brien^{3,4,5}, BA, BAI, PhD, CEng, FIEI

17

18 * both authors contributed equally to the work

19

20 **Authors' affiliations:**

21 ¹ University College Dublin Veterinary Hospital, School of Veterinary
22 Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

23 ²Mid-Atlantic Equine Medical Center, 40 Frontage Rd, Ringoes, NJ,
24 08551, the United States of America.

25 ³Tissue Engineering Research Group, Department of Anatomy, Royal
26 College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2,
27 Ireland.

28 ⁴Trinity Centre for Bioengineering, Trinity College Dublin, Dublin,
29 Ireland.

30 ⁵Advanced Materials and Bioengineering Research (AMBER) Centre,
31 RCSI & TCD, Dublin, Ireland.

32 ⁶Pferdeklinik Pegasus, Laaberstrasse 69, 2384 Breitenfurt, Austria.

33 ⁷SurgaColl Technologies Limited, Rubicon Centre, Cork Institute of
34 Technology, Rossa Avenue, Bishopstown, Cork, Ireland.

35

36 **Corresponding Author:**

37 Prof. Fergal O'Brien, Associate Professor , Tissue Engineering
38 Research Group, Department of Anatomy, Royal College of Surgeons
39 in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland.

40 Phone: +353 1 4022149 Fax: +353 1 402 2355

41 Email: fjobrien@rcsi.ie

42

43 **Place where this case was operated:**

44 University College Dublin Veterinary Hospital, Large Animal Surgery
45 Service, School of Veterinary Medicine, University College Dublin,
46 Belfield, Dublin 4, Ireland.

47

48 **Ethical approval and consent form:**

49 As this was a true clinical case and not an experimental case, no
50 ethical approval was required. Authorisation from the Irish
51 Department of Agriculture, Food and the Marine was granted to use
52 the collagen hydroxyapatite (CHA) bone graft substitute in this
53 specific case. The horse was permanently stamped "Out of the Food
54 Chain". The owner signed a consent form discharging the University
55 College Dublin Veterinary Hospital and the Royal College of Surgeons
56 in Ireland from any legal responsibilities.

57

58 **Conflict of interest:**

59 Authors John P Gleeson and Fergal J O'Brien hold IP with a
60 commercial product of related composition to the collagen-HA
61 scaffolds used in this study. SurgaColl Technologies Limited provided
62 partial financial support to the University College Dublin Veterinary
63 Hospital, School of Veterinary Medicine, University College Dublin, to
64 facilitate additional post-implantation clinical imaging of the animal.

65

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67 The cost associated with the management of this clinical case has
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74

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78 tomography

79

80

81 **Abstract (Max 250 words)**

82

83 An unmet need remains for a bone graft substitute material that is
84 biocompatible, biodegradable and capable of promoting osteogenesis
85 safely *in vivo*. The aim of this study was to investigate the use of a
86 novel collagen-hydroxyapatite (CHA) bone graft substitute in the
87 clinical treatment of a mandibular bone cyst in a young horse and to
88 assess its potential to enhance repair of the affected bone. A 2 year
89 old Thoroughbred filly, presenting with a multilobulated aneurysmal
90 bone cyst was treated using the CHA scaffold. Post-operative clinical
91 follow-up was carried out at 2 weeks and 3, 6 and 14 months.
92 Cortical thickening in the affected area was observed from CT
93 examination as early as 3 months post-surgery. At 14 months,
94 reduced enlargement of the operated mandible was observed, with no
95 fluid filled area. The expansile cavity was occupied by moderately
96 dense mineralised tissue and fat and the compact bone was
97 remodelled, with a clearer definition between cortex and medulla
98 observed. This report demonstrates the successful application of the
99 CHA scaffold material in the promotion of enhanced bone repair in
100 this craniomaxillofacial indication and thus the potential of this
101 material for translation to human applications.

102

103 **1. Introduction**

104 Segmental bone defects, occurring as a result of fractures, tumours,
105 bone cysts and other diseases, remain a significant challenge for
106 orthopaedic surgeons. Currently, the “gold standard” clinical approach
107 involves the surgical harvesting of autograft tissue, taken from the
108 patient's own body and subsequently re-implanted into the defect
109 site. However, due to the limitations associated with autograft tissue,
110 particularly in the treatment of large area defects, alternative
111 solutions are required. While tissue-derived substitutes such as
112 allografts and xenografts can offer practical advantages over
113 autograft material (e.g. no need for additional surgery, “off the shelf”
114 availability, size of graft material), their use is limited due to concerns
115 over immune reactions and transfer of host diseases.

116

117 Focus has now moved to the development of bone graft substitutes
118 and tissue engineered biomaterial scaffolds. Numerous materials are
119 currently under development, with investigators working to optimise
120 scaffold properties including biocompatibility, osteoinductivity,
121 osteoconductivity, mechanical resilience, and functional resorption
122 while minimizing inflammation and foreign body reaction (Szpalski *et*
123 *al.*, 2012). More recently, there has recently been a move towards
124 the incorporation of cells, growth factors and cellular signalling
125 molecules into these scaffold materials. In particular, the use of

126 growth factors for stimulating bone repair in challenging surgical
127 cases has become popular since the identification of bone
128 morphogenetic protein-2 (BMP-2) as an important growth factor in
129 bone formation. Recombinant versions (rhBMP-2 Infuse, Medtronic,
130 Inc (FDA, 2002) and rhBMP-7, (OP-1, Novo Noradisk (FDA, 2001))
131 have received FDA approval for specified surgical procedures, but
132 initially successful results (Sciadini and Johnson 2000; Yasko *et al.*,
133 1992) and subsequent human trials (Boden *et al.*, 2000; McKay *et*
134 *al.*, 2002) have been called into question by numerous studies citing
135 safety concerns (Garrett *et al.*, 2010; Shields *et al.*, 2006; Vaidya *et*
136 *al.*, 2009; Wong *et al.*, 2008). In addition, such is the need for a
137 viable alternative to autogenous bone that up to 85% of reported
138 BMP-2 use was off-label (Services DoHaH, 2010), thus leading to the
139 US Department of Health and Human Services calling for a review of
140 current evidence on the safety of rhBMP-2 doses and applications
141 (Services DoHaH, 2010). While this is concerning for orthopaedic
142 surgeons, it simply means that there is still an enduring and unmet
143 need for a bioactive, load-bearing tissue-engineering scaffold, which
144 is biocompatible, biodegradable and capable of facilitating and
145 promoting significant osteogenesis safely when implanted *in vivo*.

146

147 Research in the Tissue Engineering Research Group (TERG) in the
148 Royal College of Surgeons in Ireland, has led to the development of a

149 biocompatible, biomimetic and highly porous (> 95%) collagen
150 hydroxyapatite (CHA) composite scaffold (Gleeson *et al.*, 2010). The
151 CHA scaffold is fabricated by incorporating a ceramic hydroxyapatite
152 particle phase into a collagen-based scaffold using a patented mixing
153 process (O'Brien *et al.*, 2007) to produce a highly porous scaffold
154 with a composition optimised for bone repair. The osteoconductive
155 properties of the scaffold have been demonstrated *in vitro* (Gleeson
156 *et al.*, 2010). Regenerative potential has been demonstrated *in vivo*
157 in a rat calvarial defect (Gleeson *et al.*, 2010) and in load bearing
158 rabbit radial model (Lyons *et al.*, 2014). Significantly, the CHA
159 scaffold demonstrated comparable results to a collagen GAG (CG)
160 scaffold loaded with BMP2 (Lyons *et al.*, 2014). Further analysis has
161 demonstrated that the positive healing response is due to the innate
162 osteoinductivity of the scaffold as a result of the method of
163 incorporation and presentation of HA within it (Murphy *et al.*, 2014).

164

165 This study describes the use of this novel CHA bone graft substitute
166 as a viable alternative to autogenous bone in the treatment of an
167 aneurismal bone cyst in the mandible of a 2 year old Thoroughbred
168 filly. Aneurysmal bone cysts (ABCs) are rare bone lesions that can
169 affect the axial and appendicular skeleton of young animals. The
170 mandible is the most common location for ABCs in horse. In humans,
171 the metaphysis of long bones, pelvis, and vertebral column are the

172 most commonly affected areas (Cottalorda *et al.*, 2004). There is
173 currently little consensus regarding treatment options for such bone
174 lesions and their ultimate effectiveness. Curettage is commonly
175 performed and is sufficient for inactive lesions. However, the extent
176 of mandibular cortical thinning in this case raised significant concerns
177 about the use of curettage and long-term stabilisation of the tissue
178 through normal bone remodelling processes with pathological fracture
179 of the mandible posing a significant risk to the patient (Ordidge *et al.*,
180 2001). The aim of this study was to thus investigate the use of the
181 CHA bone graft substitute in this craniomaxillofacial indication and to
182 assess its potential to promote osteogenesis and cortical thickening of
183 the affected mandibular compact bone.

184

185 **2. Materials and Methods**

186 **2.1 Case Description**

187 A 2 year old Thoroughbred filly presented to University College Dublin
188 Veterinary Hospital with a large firm swelling of the right mandible of
189 unknown duration. The whole horizontal mandibular ramus was
190 enlarged, filling almost the entire intermandibular space (Fig. 1A).
191 Although no overt pain was noted under palpation, the area was
192 warm to the touch and the filly anticipated palpation. She was
193 observed to drink, eat and chew hay, her body condition was 2/5
194 (Carroll *et al.*, 1988). Routine haematology and serum biochemistry
195 were carried out and were unremarkable.

196

197 Radiography of the right mandible revealed a multiloculated
198 radiolucent expansible lesion with “soap bubble appearance”
199 extending from the mental foramen to the rostral root of the second
200 molar (M2; Triadan 410) (Fig. 1B). The compact bone was ventrally
201 thinner than normal but no periosteal reaction was noted.
202 Misalignment and distortion of the permanent teeth was noted, as
203 well as a suspicion of lysis of the 4th premolar (PM4; Triadan 408)
204 tooth bud. Computed tomography (CT) revealed a fluid (Hounsfield
205 unit (HU) 20) expansible mass in the horizontal ramus of the right
206 mandible (Fig. 1D). The expansile mass extended from the right
207 mental foramen to right mandibular M3 (Triadan 411) tooth bud (Fig.

208 1C). The mandible measured 5.5 cm at its maximal width (left
209 mandible at the same level 2.5 cm for comparison) and the mass
210 occupied 3/4 of the height of the mandible. The right mandibular
211 compact bone was thinner than normal (1-3 mm compared to 1.3-4.8
212 mm on the left mandible at the same level). It was also noted that in
213 some focal areas the cortex was perforated and the right mental
214 foramen was enlarged. The permanent teeth were distorted (mainly
215 PM3 (Triadan 407)) and/or displaced by the mass. Hypoplasia of the
216 bud of PM4 (Triadan 408) was also noted. Intraoperative aspiration of
217 the cystic fluid was performed and cytology revealed a non-septic
218 inflammation (TP=54 g/L, WBC=0.16 x10⁹/L, mainly macrophages)
219 with mild past and recent haemorrhage and mild benign osteoclast
220 proliferation. A presumptive diagnosis of a multilobulated mandibular
221 bone cyst with tooth displacement, distortion and hypoplasia was
222 made.

223

224 **2.2 Scaffold Fabrication**

225 CHA scaffolds were fabricated using a previously described freeze-
226 drying technique. Briefly, Type 1 collagen (Collagen Matrix Inc., NJ,
227 USA), hydroxyapatite ((Captal 'R' Reactor Powder, Plasma Biotal, UK)
228 and a 0.5M acetic acid solution were combined using a patented
229 blending protocol [13, 14]. The resultant CHA suspension was
230 pipetted into stainless-steel trays (internal dimensions - 60mm x

231 60mm; 18ml CHA solution per tray) and freeze-dried (Virtis Genesis
232 25EL, Biopharma, Winchester, UK) at a constant cooling rate of 1
233 °C/min to a final freezing temperature of -40°C ((Gleeson *et al.*,
234 2010; O'Brien *et al.*, 2007). Following freeze-drying, dehydrothermal
235 (DHT) treatment was carried out at a temperature of 105°C under a
236 vacuum of 0.05 bar for 24 hours (Vacucell 22; MMM, Germany).
237 Scaffolds were then chemically cross-linked for 2 hours at room
238 temperature with 1-ethyl-3-(3-dimethyl aminopropyl carbodiimide
239 (EDAC)/ N-Hydroxysuccinimide (NHS) (Sigma-Aldrich, Arklow,
240 Ireland) at a concentration of 6 mM EDAC per gram of collagen and a
241 5:2 molar ratio of EDAC:NHS (Gleeson *et al.*, 2010).

242

243 **2.3 Surgical Procedure**

244 The mare was anaesthetised and positioned in dorsal recumbency. A
245 ventromedial approach to the enlarged horizontal ramus was
246 performed via a 20 cm long skin incision. The poorly adherent
247 periosteum was reflected and the ventral surface of the bone
248 examined. Black discolorations spots were visible on the compact
249 bone surface. Two osteal windows (8 x 3 cm and 6 x 3 cm) separated
250 by a 2 cm wide bridge were created using an oscillating saw to give
251 access to the multilobulated cyst (Fig. 2B). A yellow
252 serohaemorrhagic fluid was aspirated from one of the cysts (Fig. 2A).
253 Suction was used to aspirate the remaining cystic fluid. The cyst

254 lining and bone spikes adherent to the dorsal aspect of the bone flaps
255 were curetted and flushed (Fig. 2C). Six 2 mm holes were drilled in
256 the corner/edge of the flaps and parent bone to enable flap re-
257 apposition at the end of surgery. The flaps were preserved in swabs
258 impregnated with saline and autologous venous blood and disposed in
259 a sterile kidney dish.

260

261 Several connected cystic structures were visible within the mandible
262 cavity (Fig. 2B). With assistance from the CT images, the cystic cavity
263 was debrided using a curette. The mandibular nerve was identified
264 and the debridement and curettage was initiated rostral to PM2
265 (Triadan 406). Any cystic and abnormal bone material was removed.
266 The bud of PM2 was difficult to differentiate from the cystic tissue and
267 was also debrided to remove any suspicious material. The
268 debridement was then continued around PM3 (407), PM4 (408), M1
269 (409) and M2 (410). The cavity volume was estimated by filling with
270 saline and found to be 240-250 ml in volume (Fig. 2C). The cavity
271 was then washed with saline twice and all the fluid suctioned.

272

273 As a risk of traumatic/pathologic fracture was considered high on this
274 case, CHA scaffold sheets, measuring 5 x 60 x 60 mm in dimension
275 were placed into the defect site to encourage rapid bone healing. The
276 scaffolds were positioned along the internal walls of the mandible

277 with 5 sheets inserted in total. Two CHA sheets were inserted (flat)
278 on the medial side, one (flat) on the lateral side, one caudally (rolled)
279 and one rostrally (rolled) in the cavity (Fig. 2D). The bone flaps were
280 sutured in place to the parent bone using USP 2-0 polydioxanone
281 suture material passed through drilled holes. The surface of the bone
282 was then flushed with gentamicin (500 mg) and the periosteum
283 sutured with polyglecaprone USP 2-0. A gentamicin (500mg) flush
284 was repeated and the skin apposed using skin staples and a
285 protective bandage was placed for recovery. Using a rope-assisted
286 recovery system the mare recovered from anaesthesia uneventfully.

287

288 **2.4 Post-operative Assessment**

289 Post-operative clinical re-evaluation was performed at 2 weeks and 3,
290 6 and 14 months. CT examinations were performed at 3 and 14
291 months post surgery. In order to evaluate the remodelling of the right
292 mandible following treatment, mandible bone thickness, and
293 mandible cavity area and volume measurements were carried out
294 using the OsiriX HD 4.0 software (Pixmio, Geneva, Switzerland).
295 Measurements at each time point are reported as a % size difference
296 relative to the left mandible (normal), thus accounting for any normal
297 anatomical changes resulting from growth of the animal during the
298 study. The cavity area measurements in each case were compared at
299 the widest point of the mandible.

300

301 **3. Results**

302 **3.1 Histopathology Results**

303 Tissue samples (cystic material and material coming from PM2 (406))
304 harvested during surgery were sent for histopathology (Fig. 3A). The
305 features of these samples were consistent with a multiloculated
306 aneurysmal bone cyst. The dental material submitted was consistent
307 with normal tooth root material.

308

309 **3.2 Post-operative Outcome**

310 Post-operative evaluation at 2 weeks revealed a fully healed surgical
311 wound. At 3 months post-surgery, the operated mandible appeared
312 subjectively less enlarged and the oral examination was within
313 normal limits. A CT examination revealed that most of the expansile
314 cavity was filled by moderately dense mineralised tissue (HU 200-
315 300), with a few remaining fluid filled areas (HU 10) still apparent
316 (Fig. 3C). The right mandible measured 5.6 cm at its maximal width
317 (left mandible 2.4 cm). The compact bone was continuous and was
318 generally thicker than previously described (1.2-10.7 mm compared
319 to 1.9-7.2 mm at the same levels on the left mandible). Most of the
320 tooth buds surrounding the cyst had grown but were still smaller
321 compared to the opposite side. Their displacement and distortion was
322 less severe than pre-surgery. The right mandibular PM2 tooth bud

323 showed signs of resorption of its roots. The area at the widest point
324 of the mandible was reduced compared to pre-operative
325 measurements, although the volume was slightly increased, most
326 likely due to growth of the animal (Fig. 4).

327

328 At 6 months post-surgery the horse was in training and no problems
329 with her jaw, masticatory function or bite acceptance were recorded.

330 At 14 months post-surgery, the operated mandible appeared
331 subjectively less enlarged than at 3 months. A non-painful bony
332 prominence was noted on the ventral-lateral-rostral aspect of the
333 right mandible. On oral examination the decidual PM3 (Triadan 807)
334 on the right mandible was missing while it was still firmly attached on
335 the left mandibular arcade. Eruption of the permanent PM3 (Triadan
336 407) could be palpated in the gap between PM2 (Triadan 406) and
337 PM4 (408). A repeat CT examination revealed the right mandible
338 measured 4.5 cm at its maximal width (left mandible 2.7 cm). There
339 was no fluid filled area and the expansile cavity was occupied by
340 moderately dense mineralised tissue (HU 150-300) and fat (HU 20-
341 100) (Fig. 5). The compact bone of the right mandible was
342 remodelled and a clearer definition between cortex and medulla was
343 noted. The cortex was thinner than at 3 months post-surgery (1-2.4
344 mm compared to 0.7-4.7 mm at the same levels on the left
345 mandible). The cavity area and volume measurements showed the

346 right mandible to be less enlarged than prior to surgery (Fig. 4). On
347 the right side PM2 (Triadan 406) appeared to have erupted correctly
348 but this tooth was significantly shorter than 306 (3.2 cm versus 7.4
349 cm). PM3 (Triadan 407) seemed to have just erupted. This tooth was
350 still slightly distorted with its root pointing laterally, deforming the
351 right mandible externally. Both PM4 (Triadan 408 and 308) presented
352 the same length and were covered by their dental caps. The molars
353 were symmetric between left and right and normal in appearance.

354

355 **4. Discussion**

356 The study demonstrates the successful clinical use of a collagen-
357 hydroxyapatite bone graft substitute for the treatment of an equine
358 craniomaxillofacial bone cyst. The CHA scaffold applied in this case
359 has been designed to address a major unmet need, for a bone graft
360 substitute material that is biocompatible, biodegradable and capable
361 of promoting osteogenesis safely *in vivo*. Follow-up at 3 and 14
362 months post-implantation revealed reduced enlargement of the
363 operated mandible, initial thickening of the compact bone with no
364 fluid filled area, and later remodelling of the compact bone with a
365 clearer definition between cortex and medulla. The results show the
366 potential of the scaffold to promote osteogenesis and cortical
367 thickening of the affected mandibular compact bone.

368

369 An aneurysmal bone cyst is a reasonably rare condition occurring in
370 animals (Thompson *et al.*, 2007; Bryant *et al.*, 2012) and humans
371 (Cottalorda *et al.*, 2004). Many hypotheses have been proposed to
372 explain the etiology and pathogenesis of aneurysmal bone cysts
373 (Jaffe *et al.*, 1942; Lichtenstein *et al.*, 1957). One of the more
374 commonly accepted ideas that increased venous pressure and a
375 resultant dilation and rupture of the local vascular network could
376 trigger onset of the cystic growth (Jaffe *et al.*, 1942). The lesion may
377 be primary with possible genetic predisposition (Leithner *et al.*,
378 2004), or secondary to a pre-existing lesion such as fibrous dysplasia,
379 hematoma from trauma, bleeding disorders, or within a pre-existing
380 bone tumor (Leithner *et al.*, 2004). Giant cell tumors are the most
381 common cause in humans (Wu *et al.*, 2011). In the case of the
382 presenting filly, no bleeding disorder was identified pre-operatively
383 and no bone tumor was observed on histopathological analysis. No
384 previous trauma was reported although this could not be totally
385 excluded as her early history was undocumented. The potential
386 genetic predisposition of her family to bone lesions was not
387 investigated. The etiology of this lesion remains unclear in this
388 patient.

389

390 Absolute alcohol intracystic injection has been reported to be
391 successful in the management of aneurysmal bone cysts in humans

392 (Cottalorda *et al.*, 2004). However, due to the multiloculated
393 appearance of the cyst, the proximity of the tooth roots/buds and
394 mandibular nerve, this non-invasive option was considered too risky.
395 Autologous cancellous bone grafting was also considered a poor
396 option due to the size of the cavity. The use of a synthetic bone graft
397 thus provided an ideal solution. The technique employed in this case
398 involved surgical curettage to remove the cyst followed by
399 implantation of a tissue engineered scaffold to encourage repair of
400 the mandible bone. This is the first time that this combination of
401 techniques has been used to treat a large aneurysmal bone cyst. Due
402 to the high risk of traumatic or pathologic mandibular fracture in this
403 case, during surgery particular attention was paid to apply the
404 scaffold to the area where the compact bone was extremely thin or
405 perforated. Two additional sheets of the CHA bone graft substitute
406 were also rolled and placed cranially and caudally to provide some
407 healing in the cavity itself and underneath the bone flaps. The CHA
408 scaffold displayed a perfect ability to adhere to the compact bone and
409 demonstrated sufficient mechanical strength, flexibility and durability
410 to withstand surgical handling and to be rolled and shaped to fit into
411 the required spaces within the mandible cavity. The bone fenestration
412 technique with the central bridge provided stability of this weakened
413 mandibular bone during the curettage and early post-operative
414 period. Although the blood supply to the bone flaps was completely

415 absent for the first days after surgery, no bone necrosis was noted
416 and proper healing was evident on CT examination performed 3
417 months post-surgery. This maximum bone preservation approach
418 likely contributed to the good recovery of this mare.

419

420 Bone repair was quantified at 3 months and 14 months post-surgery.
421 The amount and quality of compact bone produced in 3 months was
422 considered exceptional on comparative CT examinations. Analysis of
423 the CT images revealed no trace of the CHA scaffold thus
424 demonstrating the biodegradability of the scaffold and its successful
425 resorption by the body as new repair tissue is formed. Prior to
426 surgery, growth of the tumor within the mandible led to enlargement
427 of mandible and thinning of the mandibular compact bone leaving the
428 horse at significant risk of pathological fracture. Evaluation at 3
429 months confirmed that rapid repair of the mandible bone had
430 occurred. Importantly, the compact bone was found to be continuous,
431 with increased thickness compared to pre-surgery values and thus
432 the risk of pathological fracture of the mandible was significantly
433 reduced.

434

435 At 14 months, CT analysis revealed that further remodelling of the
436 compact bone of the right mandible had occurred and a clearer
437 definition between cortex and medulla was noted. This was confirmed

438 through mandible volume and area measurements, with the cavity
439 volume being significantly reduced at 14 months compared to pre-
440 surgery values. Importantly, no reoccurrence of the cyst was
441 observed up to 14 months post-surgery. Oral examination revealed
442 that on the right side, PM2 was significantly shorter than the left. It is
443 likely the dental bud of PM2 was damaged during the curettage of the
444 most rostral part of the cavity. Although curettage was carried out
445 carefully with the use of a narrow suction tip to remove the fibrous
446 tissue lining cystic material can be easily confounded with dental buds
447 during surgery. While PM2 will require further monitoring it is unlikely
448 to cause any significant issues.

449

450 These results demonstrate the benefits of the osteoinductive
451 properties provided by the hydroxyapatite component of the scaffold
452 combined with the biocompatibility and rapid degradation associated
453 with the collagen component of the CHA scaffold. Notably, the bone
454 remodelling observed here was achieved in the absence of additional
455 osteogenic factors confirming the previously demonstrated
456 osteoconductive and osteoinductive properties of this CHA scaffold
457 and its ability to lead to tissue regeneration without the requirement
458 for addition of growth factors such as BMP (Gleeson *et al.*, 2010;
459 Lyons *et al.*, 2014; Murphy *et al.*, 2014). This off-the-shelf, cell-free
460 approach overcomes many of the limitations associated with currently

461 used autologous bone grafting procedures providing an ideal
462 alternative from a clinical and regulatory stand-point.

463

464 5. Conclusion

465 This case study investigated the use of the CHA scaffold in the
466 treatment of a multilobulated aneurysmal bone in a young horse and
467 demonstrated its potential to enhance repair of the affected bone.
468 Clinical follow-up at 3 and 14 months post-implantation revealed
469 reduced enlargement of the operated mandible, initial thickening of
470 the compact bone with no fluid filled area, and later remodelling of
471 the compact bone with a clearer definition between cortex and
472 medulla. Overall, the successful clinical outcome and enhanced bone
473 formation observed in this craniomaxillofacial indication demonstrates
474 the potential of this bone graft substitute for use in this and other
475 equine indications and also for translation to human applications.

476

477 **References**

478 Baxter GM, Turner SA. 2002, 'Diseases of bone and related
479 structures' in *Adams' lameness in horses (5th edition)*, eds. Stashak
480 ST, Lippincott Williams & Wilkins, Baltimore, MD; 433-4

481 Boden SD, Zdeblick TA, Sandhu HS, Heim SE. 2000, The use of
482 rhBMP-2 in interbody fusion cages. Definitive evidence of
483 osteoinduction in humans: a preliminary report, *Spine*, **25**: 376-381

484 Bryant U, Fallon L, Lee M, Pool R. 2012, Congenital Aneurysmal Bone
485 Cyst in a Foal. *J Equine Vet Sci*, **32**: 320-323

486 Carroll CL, and Huntington PJ. 1988, Body Condition Scoring and
487 Weight Estimation of Horses. *Equine Vet J*, **20**: 41-45

488 Cottalorda J, Kohler R, de Gauzy JS, Chotel F, Mazda K, Lefort
489 G, Louahem D, Bourelle S, Dimeglio A. 2004, Epidemiology of
490 aneurysmal bone cyst in children: a multicenter study and literature
491 review, *J Pediatr Orthop*, **13**: 389-94

492 FDA . 2002, InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion
493 Device - P000058. United States Food and Drug Administration.
494 Available from:
495 http://www.accessdata.fda.gov/cdrh_docs/pdf/P000058a.pdf [2nd
496 September 2014]

497 FDA. 2001, OP-1™ - H010002. United States Food and Drug
498 Administration. Available from:
499 http://www.accessdata.fda.gov/cdrh_docs/pdf/h010002a.pdf. [2nd
500 September 2014]

501 Garrett MP, Kakarla UK, Porter RW, Sonntag VK. 2010, Formation of
502 painful seroma and edema after the use of recombinant human bone
503 morphogenetic protein-2 in posterolateral lumbar spine fusions,
504 *Neurosurgery*, **66**: 1044-1049

505 Gleeson JP, Plunkett NA, O'Brien FJ. 2010, Addition of hydroxyapatite
506 improves stiffness, interconnectivity and osteogenic potential of a
507 highly porous collagen-based scaffold for bone tissue regeneration,
508 *Eur Cell Mater*, **20**: 218-230

509 Jaffe JL, Lichtenstein L. 1942, Solitary unicameral bone cyst with
510 emphasis on the roentgen picture. *Arch Surg*, **44**: 1004-25

511 Leithner A, Machacek F, Haas OA, Lang S, Ritschl P, Radl R,
512 Windhager R. 2004, *J Pediatr Orthop B*, **13**: 214-7

513 Lichtenstein L. 1957, Aneurysmal bone cyst: Observations on 50
514 cases. *J Bone Joint Surg Am*, **3**: 837-882

515 Lyons F, Gleeson JP, Partap S, O'Brien FJ. 2014, Novel micro-
516 hydroxyapatite particles in a collagen scaffold: a bioactive bone void
517 filler? *Clin Orthop Relat Res*, **472**: 1318-28

518 McKay B, Sandhu HS. 2002, Use of recombinant human bone
519 morphogenetic protein-2 in spinal fusion applications, *Spine*, **27**:
520 S66-85

521 Murphy CM, Schindeler A, Gleeson JP, Yu NY, Cantrill LC, Mikulec K,
522 Peacock L, O'Brien FJ, Little DG. 2014, A collagen-hydroxyapatite
523 scaffold allows for binding and co-delivery of recombinant bone
524 morphogenetic proteins and bisphosphonates, *Acta Biomater*, **10**:
525 2250-8

526 O'Brien FJ, Plunkett NA, Gleeson JP. 2007, A collagen/hydroxyapatite
527 composite scaffold, and process for the production thereof, US Patent
528 8,435,552 WO Patent 2008096334A2

529 Ordidge R. 2001, Pathological fracture of the radius secondary to an
530 Aneurysmal bone cyst in a horse, *Eq Vet Educ*, **13**: 239-242

531 Sciadini MF, Johnson KD. 2000, Evaluation of recombinant human
532 bone morphogenetic protein-2 as a bone-graft substitute in a canine
533 segmental defect model, *J Orthop Res*, **18**: 289-302

534 Services DoHaH. 2010, Bone Morphogenetic Protein: The State of the
535 Evidence of On-Label and Off-Label Use. www.hhs.gov. Available
536 from:
537 http://www.cms.gov/DeterminationProcess/downloads/id75ta.pdf?bcsi_scan_9CF786ACA806128D=0&bcsi_scan_filename=id75ta.pdf [2nd
538
539 September 2014]

540 Shields LB, Raque GH, Glassman SD, Campbell M, Vitaz T, Harpring J,
541 Shields CB. 2006, Adverse effects associated with high-dose

542 recombinant human bone morphogenetic protein-2 use in anterior
543 cervical spine fusion, *Spine*, **31**: 542-547

544 Szpalski C, Wetterau M, Barr J, Warren SM. 2012, Bone tissue
545 engineering: current strategies and techniques—Part I: Scaffolds,
546 *Tissue Eng Part B*, **18**: 246-57

547 Thompson KG. 2007, 'Diseases of bones' in *Jubb, Kennedy, and*
548 *Palmer's pathology of domestic animals* (5th ed), eds. Maxie MG,
549 Saunders, Philadelphia, PA; 129

550 Vaidya R. 2009, Transforaminal interbody fusion and the "off label"
551 use of recombinant human bone morphogenetic protein-2, *Spine J*, **9**:
552 667-669

553 Wong DA, Kumar A, Jatana S, Ghiselli G, Wong K. 2008, Neurologic
554 impairment from ectopic bone in the lumbar canal: a potential
555 complication of off-label PLIF/TLIF use of bone morphogenetic
556 protein-2 (BMP-2), *Spine J*, **8**: 1011-1018

557 Wu Z, Yang X, Xiao J, Feng D, Huang Q, Zheng W, Huang W, Zhou Z.
558 2011, Aneurysmal bone cyst secondary to giant cell tumor of the
559 mobile spine: a report of 11 cases, *Spine*, **36**: 1385-90

560 Yasko AW, Lane JM, Fellingner EJ, Rosen V, Wozney JM, Wang EA.
561 1992, The healing of segmental bone defects, induced by
562 recombinant human bone morphogenetic protein (rhBMP-2). A

563 radiographic, histological, and biomechanical study in rats, *J Bone*
564 *Joint Surg Am*, **74**: 659-670

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