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Design and evaluation of 3D-printed Sr-HT-Gahnite bioceramic for FDA regulatory submission: A Good Laboratory Practice sheep study☆

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ABSTRACT

There is an unmet clinical need for a spinal fusion implant material that recapitulates the biological and mechanical performance of natural bone. We have developed a bioceramic, Sr-HT-Gahnite, which has been identified as a potential fusion device material. This material has the capacity to transform the future of the global interbody devices market, with follow on social, economic, and environmental benefits, rooted in its remarkable combination of mechanical properties and bioactivity. In this study, and in line with FDA requirements, the *in vivo* preclinical systemic biological safety of a Sr-HT-Gahnite interbody fusion device is assessed over 26 weeks in sheep under good laboratory practice (GLP). Following the in-life phase, animals are assessed for systemic biological effects via blood haematology and clinical biochemistry, strontium dosage analysis in the blood and wool, and histopathology examination of the distant organs including adrenals, brain, heart, kidneys, liver, lungs and bronchi, skeletal muscle, spinal nerves close to the implanted sites, ovaries, and draining lymph nodes. Our results show that no major changes in blood haematology or biochemistry parameters are observed, no systemic distribution of strontium to the blood and wool, and no macroscopic or histopathological abnormalities in the distant organs when Sr-HT-Gahnite was implanted, compared to baseline and control values. Together, these results indicate the systemic safety of the Sr-HT-Gahnite interbody fusion device. The results of this study extend to the systemic safety of other Sr-HT-Gahnite implanted medical devices in contact with bone or tissue, of similar size and manufactured using the described processes.

Statement of significance

This paper is considered original and innovative as it is the first that thoroughly reports the systemic biological safety of previously undescribed bioceramic material, Sr-HT-Gahnite. The study has been performed under good laboratory practice, in line with FDA requirements for assessment of a new interbody fusion device, making the results broadly applicable to the translation of sheep models to the human cervical spine; and also the translation of Sr-HT-Gahnite as a biomaterial for use in additional applications.

We expect this study to be of broad interest to the readership of Acta Biomaterilia. Its findings are directly applicable to researchers and clinicians working in bone repair and the development of synthetic biomaterials.

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1. Introduction

Globally, bone and joint-related degenerative problems are a major cause of half of all chronic diseases in people aged over 50 [1]. Bone graft products are used widely in surgical orthopaedic applications to prevent movement in the spine, repair injured bone/joints, accelerate repair of fractures with bone loss, or as bone void fillers. Bone grafting for spinal fusion accounts for over half of all bone grafting procedures [2]. In 2017 the global interbody fusion cage market accounted for USD 1.89 Billion and is estimated to grow at a compound annual growth rate (CAGR) of 3.4% from 2018 to 2023, to USD 2.30 Billion. While market growth is driven by ageing of the population and incidence of spinal and sports injuries, it is hampered by the high cost of spinal fusion surgeries, lack of trained professionals, and stringent rules and regulations for using such devices [3].

The key shortcoming of clinically available ceramic synthetic bone graft substitutes is that they are unable to both withstand significant mechanical loads and concurrently integrate into the bone structure, so are thus restricted in their application to bone fillers and non-load-bearing applications [4]. Current ceramic synthetic bone substitutes are therefore not suitable for use in the load bearing spinal column. Existing spinal cage devices are manufactured from permanent, load-bearing materials such as polyetheretherketone (PEEK) and titanium [5]. However, clinically available spinal fusion devices offer minimal bone integration [6,7], undergo regular subsidence and migration [8,9], contribute to infection and inflammation [10,11], and require bone grafting, bringing additional surgical risk and hospital time [12] and ongoing health complications [13]. There is an unmet clinical need to develop a spinal fusion implant material that duplicates the performance of natural bone, achieving the critical combination of mechanical properties required for load-bearing applications and bioactivity required for bone regeneration and integration.

To address this challenge, we developed a multi-component ceramic comprising strontium-doped Hardystonite ($Ca_2ZnSi_2O_7$) and Gahnite ($ZnAl_2O_4$), hereafter called Sr-HT-Gahnite, (Sr–$Ca_2ZnSi_2O_7$–$ZnAl_2O_4$). To date, we have demonstrated that Sr-HT-Gahnite scaffolds possess exceptional properties in compressive strength and toughness and have outstanding bone regeneration ability without the need for supplementary bone grafting [14–20]. With no current competitor device delivering these properties concurrently, Sr-HT-Gahnite has the potential to transform the future of the USD 2.3 Billion global interbody devices market, with human, economic, and environmental benefits.

To progress this Sr-HT-Gahnite technology towards commercial outcomes, and in line with FDA requirements we test the safety of 3D printed Sr-HT-Gahnite in an *in vivo* GLP large preclinical sheep model. Development of the ceramic 3D-printing process for an approved off-the-shelf cervical interbody fusion device may facilitate future tailoring of the medical devices for personalised use. Since the architecture and surface topography of 3D-printed devices is specific to the manufacturing method and influence the material properties, specific assessment of the safety profile with respect to the 3D-printing method is required. The study endpoints cover systemic biological safety evaluations of clinical pathology blood analysis, systemic strontium distribution via blood and wool dosage testing, necropsy and macroscopic examinations, and histopathological analysis of the organs. This study was based on ISO 10,993–6, Biological Evaluation of Medical Devices, Part 6 (2016): Tests for Local Effects after Implantation; Guidance for Industry and FDA Staff: Class II Special Controls Guidance Document: Intervertebral Body Fusion Device [21]; and ASTM F2884, Standard Guide for Pre-Clinical *in vivo* Evaluation of Spinal Fusion. We demonstrate absence of any deleterious systemic distant tissue effects, including no systemic distribution of strontium in the blood and wool, indicating the systemic safety of Sr-HT-Gahnite devices in spinal implantation. This study supports continued clinical investigation of Sr-HT-Gahnite spinal devices, including evaluation of implant performance and local biological safety.

2. Materials and method

2.1. Study design

Eighteen skeletally mature female sheep (weight 56 to 79 kg, age 2.3 to 4.0 years, breed Blanche du Massif Central) underwent anterior cervical discectomy and fusion at two non-adjacent cervical disc levels (C2/C3 and C4/C5 levels) and were evaluated at 26 weeks. The cervical spine fusion involved the implantation of an interbody fusion device (test or control article). In addition, the flank of each animal was implanted with one subcutaneous implant, to increase overall exposure for evaluation of systemic effects, thereby each animal representing one experimental unit.

The sheep model was selected because it is a well-established model for similar studies; the ovine and human cervical spines have similar properties regarding anatomy and biomechanics [22–25]. The implant is intended for use in humans between the C2-C3 disc space and the C7-T1 disc space, with a maximum of two devices implanted per patient. To match the maximum of two cervical implants, as well as accounting for practical limitations in the sheep model of restricted access below the C5 vertebrae, two cervical implantations were performed.

The subcutaneous implantation allowed a three-device implantation (two cervical, one subcutaneous) scheme to ensure an exaggerated worst-case exposure for evaluation of strontium in blood and wool concentration, since only a maximum of two devices could be accommodated in the sheep cervical spine. Strontium was also dosed in wool because trace metals are incorporated into the wool structure through diet and exposure to exogenous sources.

Sheep were implanted with either the test article (Sr-HT-Gahnite Cervical Fusion Cage, Allegra Orthopaedics) or the control article (Valeo C, CTL Amedica Corporation), with eight animals in each group plus two reserve animals implanted with the test device to cover cases of premature euthanasia due to the long-term nature of the study. The systemic effect of each article was evaluated through (1) blood biochemistry analysis and complete blood count, (2) strontium dosage in blood and wool, and (3) histopathologic evaluation of distant organs. Blood analyses were performed throughout the study to assess the general health of the animals. An analysis of blood and wool strontium levels was done to evaluate systemic distribution of strontium from the test article.

2.2. Fabrication and identification of study materials

2.2.1. Fabrication of test article

The test article was *Sr-HT-Gahnite Cervical Fusion Cage*, manufactured by 3D printing and provided by Allegra Orthopaedics (Fig. 1); a single-component device with a solid outer wall and internal porous core that supports bone growth. It functions as a physical scaffold to bear load and support bone ingrowth during vertebral fusion; no use of supplementary bone graft was required. Precursor ceramic powder for producing 3D printed Sr-HT-Gahnite scaffolds was prepared using the sol-gel method, with aluminium oxide (Al_2O_3) powder, as previously described [14]. The powder was ground using a ball mill machine (Across International, Australia) to obtain median particle sies of 5 µm (Horiba, Japan). Test articles with outer dimensions of 12 mm (D) x 16 mm (W) x 5 mm (H) and parallel superior/inferior surfaces were produced by stereolithography, with controlled heat treatment to decompose the organic material in the scaffolds followed by heating up to 1230 °C

Fig. 1. Test article, Sr-HT-Gahnite Cervical Fusion Cage, Allegra Orthopaedics.

Fig. 2. Control article, Valeo C, CTL Amedica Corporation.

to sinter the ceramic particles. Articles were sterilised by ethylene oxide.

The test article implanted subcutaneously measured 14 mm (D) x 18 mm (W) x 10 mm (H), with parallel surfaces. The test article represents the final form intended for clinical use, so no supplemental bone graft was utilized.

2.2.2. Identification of control article

The control article was *Valeo C*, manufactured and provided by CTL Amedica Corporation (Fig. 2). Valeo C features a convex, bullet nose design and an axial void designed to hold bone graft material. The devices are designed with angular teeth to allow the implant to grip the superior and inferior verterbral end plates, thus resisting expulsion. Manufactured from MC2 ceramic material (silicon nitride) and sterilised by gamma irradiation, the control articles were FDA-approved, clinically available devices. According to the instructions for use, the control article was packed with bone autograft, sampled on the iliac crest of the sheep.

The control article implanted in the cervical spine measured 12 mm (D) x 16 mm (W) x 5 mm (H), with parallel superior/inferior surfaces. The test article implanted subcutaneously measured 14 mm (D) x 17 mm (W) x 10 mm (H), with a 6-degree lordosis angle.

2.2.3. Supplemental fixation

The test and control articles were secured in the intervertebral space using an anterior fixation plating system, sterilized by steam at 134 °C for 18 min. Fixation plates were 1-level, 24 mm or 26 mm in length while fixation screws measured 4.0 x 15 mm (NuVasive, Australia).

2.3. Test facility, housing and diet

The study was performed at NAMSA, Chasse-sur-Rhône, France. NAMSA is an AAALAC international accredited facility and is registered with the French Department of Agriculture for animal housing, care, and investigations. Conditions conformed to the European requirements on the protection of animals used for scientific purposes (EU Directive 2010/63/EU). Animals were housed in groups under laboratory conditions, with temperature fixed to 15 - 24 °C and the light cycle controlled using an automatic timer (12 h of light, 12 h of dark). Standard hay was provided *ad libitum* and supplemented with a commercially available pelleted sheep feed (Special Diet Services, France). Minerals were provided *ad libitum* (Sodimouton, Salins Agriculture). Potable water was delivered *ad libitum* through species appropriate containers or delivered through an automatic watering system. No contaminants present in the feed or water were expected to adversely impact the results of this study.

2.4. Animal preparation

Sheep were enrolled into the study based on minimum weight of 50 kg at implantation, and age of 2.0 to 4.0 years, with skeletal maturity confirmed. Sedation, analgesia, anaesthesia, and antibiotic treatments were administered pre-, intra-, and post-operatively as required and according to standard animal care. Each sheep was intubated, mechanically ventilated, and electrocardiogram (ECG), peripheral non-invasive arterial blood pressure and oxygen saturation were monitored. The surgical areas were clipped free of wool, scrubbed with povidone iodine (Vetedine savon®, Vetoquinol), wiped with 70% isopropyl alcohol (Savetis), painted with povidone iodine solution (Vetedine solution®, Vetoquinol) and draped. The sheep were placed in the lateral or supine position (for the subcutaneous or cervical implantation, respectively) on a warmed pad. The neck of the sheep was maintained using an appropriate support to minimise movement during the cervical implantation procedure. A rectal temperature probe and a rumen tube were placed during surgery. Two implantation procedures were performed each day, one test group and one control group, with the order alternated daily to account for surgical or environmental factors.

2.5. Anterior cervical discectomy and fusion (ACDF) procedure

For the control article, bone autografts were harvested from the right iliac crest of each animal before starting the cervical implantation and stored on a saline soaked gauze to prevent drying. Upon completion of the procedures, incisions were closed using standard surgical technique and disinfected using povidone iodine solution (Vetedine solution®, Vetoquinol).

A single skin incision was made on the flank of the sheep to implant a device and also harvest bone as necessary. A pocket large

enough to accommodate each test article or control article (without bone autograft) was formed by blunt dissection in the subcutaneous tissue. The article was entirely introduced into the pocket. The skin was closed and disinfected.

The Smith-Robinson style approach was used to access the anterior surface of the cervical disc spaces to be implanted. The levels of the exposed cervical intervertebral discs were confirmed with fluoroscopy. Each cervical disc level to be implanted was operated separately, and a Caspar distractor was placed for intervertebral distraction. A discectomy was performed at each implant site and the endplates of the adjacent vertebral bodies were rasped and burred (5.0 mm drill, Stryker®) to accommodate the article while maintaining the posterior longitudinal ligament. Animals were implanted with a test or control article at two nonadjacent cervical disc levels (C2/C3 and C4/C5). Surgeon blinding was not possible due to visible differences between the test and control devices.

After implantation, the vertebral distractor was released, and the anterior aspect of the vertebral bodies were prepared to provide an even surface for placement of a cervical plate. Test and control articles were maintained within the intervertebral space with a 1-level fixation plate and two pairs of screws fixed in the adjacent vertebrae. Immediately after implantation, radiographs of the cervical spine were performed (fronto-dorsal and lateral views) and the position of the article was validated by the surgeon. Upon completion of the ACDF procedure, skin and muscles were closed using standard surgical technique.

2.6. Clinical observations

An individual post-operative follow-up of each animal was conducted daily during the postoperative period for 14 days post-surgery and then once a week until week 6. This post-operative examination included but was not limited to paresis, paralysis, pain on palpation of the surgical sites, swelling of the surgical sites, abnormal neck position, and dysphagia. The implantation sites were examined daily for adverse reactions until sutures and staples removal after complete healing (approximately 2 weeks following surgery). A detailed clinical examination including general behaviour and appearance, locomotion, posture, gastro-intestinal, urinary, respiratory, cardiovascular system observations, abdominal, cutaneous, subcutaneous, ocular, buccal, nasal, and genital organs and homoeostasis observations of the animals was conducted at least once every two weeks. Body weights were recorded to the nearest whole kilogram every two weeks for the first 6 weeks, then monthly.

2.7. Blood and wool sampling

Venous blood was sampled prior to implantation (Day 0), at 4, 8, 13 and 20 weeks after implantation and at termination (Week 26) for complete blood count (CBC), biochemistry and strontium concentration analysis. Blood samples for strontium concentration analysis were collected in EDTA K_3 and dry tubes, stored between −25 °C to −15 °C. Blood samples for biochemistry analysis were collected in dry tubes, stored at 15 °C to 25 °C, and blood samples for complete blood count were collected in EDTA K_3 tubes, stored at 15 °C to 25 °C.

Wool was sampled before implantation and at termination for strontium concentration analysis. Wool was collected from the subcutaneous implantation area (a minimum area of approximately 20 x 20 cm), weighed, and stored at room temperature (15 °C to 25 °C).

Due to the limitations on sample analysis under GLP validated conditions, samples collected from $n = 6$ of the $n = 10$ available test animals, and $n = 4$ of the $n = 8$ available control animals were selected for strontium analysis at each timepoint. Testing operators were blinded to the experimental group of each animal.

2.8. Terminal procedure

At week 26 following implantation, the animals were weighed and euthanized by an intravenous injection of a lethal solution (Doléthal®, Vetoquinol). Final radiographs of the whole cervical spine were performed (fronto-dorsal and lateral views). The subcutaneous implanted sites were macroscopically examined, collected, and fixed in 10% neutral buffered formalin (NBF, VWR) for storage. The animals were placed in supine position to allow removal of the ventral neck skin and head, and sampling of the mandibular lymph nodes. Samples were excised from the brain and the axillary and caudal deep cervical draining lymph nodes. Then the trachea and oesophagus were removed to expose the implanted cervical sites. The neck was removed from the body between C7 and T1 vertebrae. The spinal nerves and muscle were consistently sampled as close as possible to the implanted sites. Radiographs were performed to localize the C4/C5 vertebrae, and the neck was sectioned between these two vertebrae. The muscle was gently dissected from the C2/C3 and C4/C5 implanted sites.

The implanted sites were macroscopically examined and photographed, and any gross changes in tissues surrounding each article were recorded using the following parameters: size, shape, colour, consistency, distribution, and any other observations, as appropriate. Macroscopic implant stability, and the presence of implant and material debris were described. The two cervical sites with the vertebral bodies above and below each article were orientated. The C2/C3 implanted sites were fixed in 10% neutral buffered formalin (VWR, USA) for histopathologic analysis. The C4/C5 implanted sites were harvested and kept frozen (−25 °C to −15 °C) but not further evaluated. Organs (adrenals, brain, heart, kidneys, liver, lungs and bronchi, skeletal muscle, spinal nerves close to the implanted sites, ovaries, and draining lymph nodes) were macroscopically examined, sampled, and fixed in 10% NBF for histopathologic analysis. Since the two reserve test animals were not required to be used as replacement animals, all $n = 10$ test animals were available for analysis.

2.9. Clinical pathology

Blood biochemistry and complete blood count were analysed for all animals, $n = 10$ for the test group and $n = 8$ for the control group. Analysis parameters for haematology complete blood count were haemoglobin, total white blood cell count, and differential white blood cell count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) – absolute and relative counts. Analysis parameters for clinical biochemistry were sodium, potassium, chloride, calcium, inorganic phosphorus, blood urea nitrogen, creatinine (serum), total cholesterol, triglycerides, total bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase. Testing operators were blinded to the experimental group of each animal.

2.10. Histopathology

After complete fixation in 10% NBF (VWR), lymph nodes, organs and anomaly samples were dehydrated in alcohol solutions of increasing concentration, cleared in xylene, and embedded in paraffin. One central transverse section per block was cut with a microtome (4.5 µm thickness) and stained with safranin-hematoxylin-eosin (SHE).

Organs and draining lymph nodes underwent qualitative and semi-quantitative histopathological analysis for any article-related

morphological changes, potential degradation particles and systemic effects. Histopathologic findings of the sampled organs were graded in severity using a five-point system of minimal, slight, moderate, marked, or severe according to the rules of General Pathology. Representative photomicrographs were taken of the areas of interest. Due to the limitations on sample analysis under GLP validated conditions, samples collected from $n = 6$ of the $n = 10$ available test animals, and $n = 4$ of the $n = 8$ available control animals were selected for histopathological analysis. Blinding was not possible due to visible differences between the test and control devices.

2.11. Statistical analysis

The test article was evaluated and compared to the control article. All data have been expressed as mean ± standard deviation, along with the individual data points, unless otherwise stated. Statistical significance was evaluated for two groups using a two-tailed Student's *t*-test, with normality assumed. P-value < 0.05 was considered statistically significant.

3. Results

3.1. Operative and post-operative observations indicate tolerance of the articles

The bone autograft harvesting, subcutaneous implantation and cervical implantation of the articles were performed without difficulty. The radiographs performed at the end of implantation showed no abnormality and good placement of the articles and plates/screws (Fig. 3).

Individual body weight data are shown in Table 1. Globally, all sheep gained weight or had a stable weight throughout the study, indicating satisfactory health throughout implantation.

Transitory swelling of the cervical sites was observed in 5/8 (62%) of the control sheep and 2/10 (20%) of the test sheep. This swelling was related to the surgical procedure including device implantation, appearing between Day 1 and Day 13, and usually lasted one to five days. The swelling was attributed to the surgery as they were observed with the same severity and frequency in the test and control sheep ($p > 0.05$), likely affected by the limitations of assessing swelling via gross examination. Clinical abnormalities such as pneumonia, liver parasites, and presence of bacterial colonies in the lungs, were deemed incidental and not related to the article implantation, since they are background diseases commonly found in farm sheep.

3.2. Clinical pathology parameters demonstrate absence of infection indicators

There were no major changes in haematology parameters at termination after implantation of the test articles when compared to baseline and control values (Table 2). Data for timepoints throughout implantation are shown for haemoglobin (Fig. 4) and total white blood cell count (Fig. 5). All data variations between test and control groups at each timepoint, as well as between baseline (day 0) and each timepoint for both test and control groups, are not statistically significant at $p<0.05$.

3.3. Blood clinical biochemistry parameters remain stable throughout implantation

There were no major changes in clinical chemistry parameters after implantation of the test articles when compared to baseline and control values (Table 3).

3.4. Presence of test article does not affect strontium concentration in blood or wool

Blood samples were analysed for strontium concentration and the results are shown in Fig. 6. The presence of test article did not affect strontium concentration in blood. All data variations between baseline (day 0) and each timepoint value for the test group

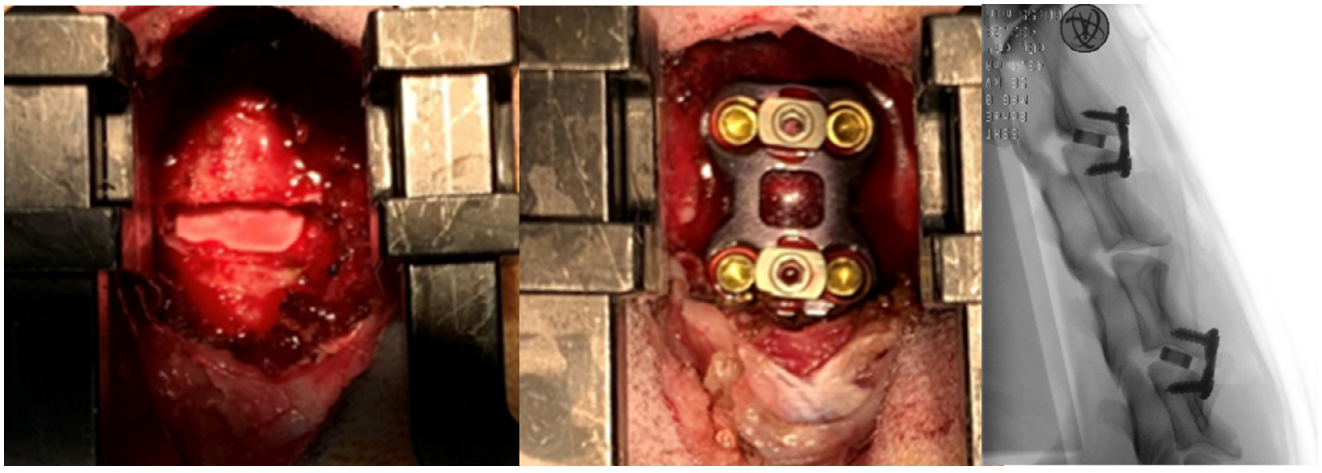

Fig. 3. C2/3 Implantation of test article prior to applying supplemental fixation (left), final implantation including supplemental fixation prior to site closure (centre), radiograph of C2/3 and C4/5 implantation sites (right).

Table 1
Body Weight at implantation and termination. Data expressed as mean ± standard deviation.

Group	Body Weight at Implantation (kg)	Body Weight at Termination (kg)	Body Weight Variation between Termination and Implantation	
			kg	%
Control	70 ± 7	78 ± 7	8 ± 2	12 ± 4
Test	65 ± 6	68 ± 8	3 ± 4	4 ± 6

Table 2
Haematology parameters at implantation and termination. Data expressed as mean ± standard deviation.

Haematology Parameter	Units	Test		Control	
		Day 0 (Implantation)	Week 26 (Termination)	Day 0 (Implantation)	Week 26 (Termination)
Haemoglobin (HB)	g/L	125 ± 14	120 ± 14	128 ± 13	118 ± 5
Total White Blood Cell Count (WBC)	giga/L	6.15 ± 3.05	4.98 ± 1.45	6.90 ± 1.29	5.33 ± 0.92
Neutrophils	% WBC	53.3 ± 16.0	59.4 ± 16.0	61.1 ± 10.5	45.7 ± 10.4
(PCN)	giga/L	3.47 ± 2.49	2.99 ± 1.24	4.29 ± 1.36	2.47 ± 0.81
Eosinophils	% WBC	6.4 ± 3.1	2.4 ± 1.3	6.3 ± 2.0	3.3 ± 2.2
(PCE)	giga/L	0.41 ± 0.32	0.12 ± 0.07	0.43 ± 0.14	0.17 ± 0.09
Basophils	% WBC	0.4 ± 0.8	0.0 ± 0.0	0.2 ± 0.3	0.2 ± 0.4
(PCB)	giga/L	0.02 ± 0.04	0.00 ± 0.00	0.02 ± 0.03	0.01 ± 0.02
Lymphocytes	% WBC	35.1 ± 12.7	35.3 ± 15.2	29.6 ± 10.0	47.9 ± 11.0
	giga/L	1.99 ± 0.89	1.74 ± 0.92	1.97 ± 0.61	2.54 ± 0.69
Monocytes	% WBC	4.8 ± 4.3	2.9 ± 2.5	2.8 ± 1.4	2.7 ± 2.8
	giga/L	0.25 ± 0.18	0.13 ± 0.10	0.19 ± 0.09	0.13 ± 0.12

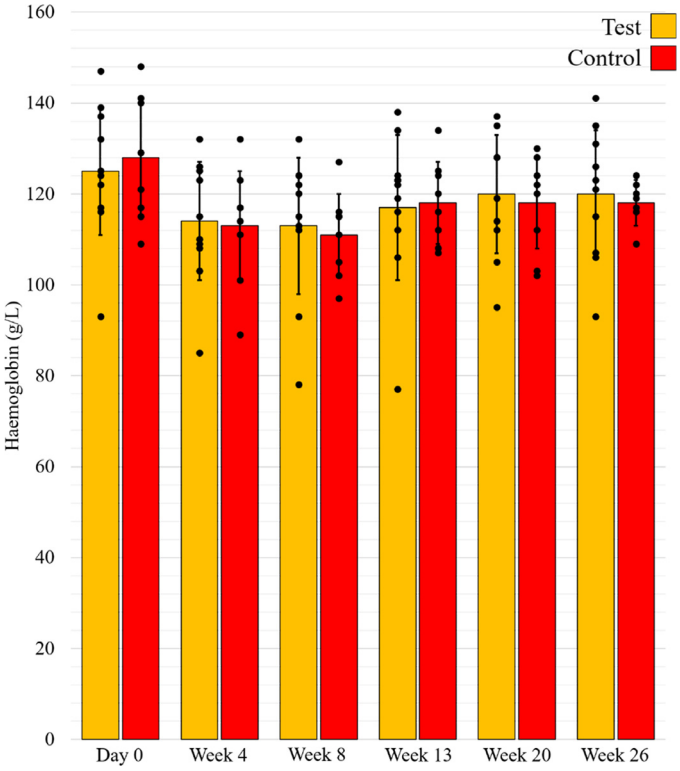

Fig. 4. Blood Haemoglobin content throughout implantation.

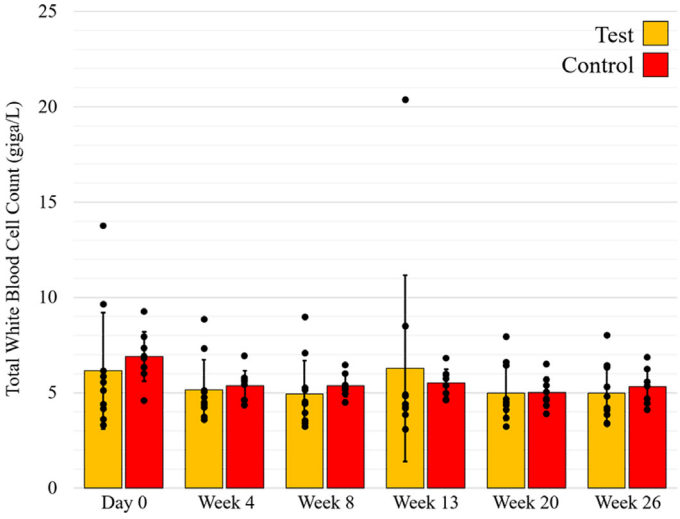

Fig. 5. Blood total white blood cell count throughout implantation.

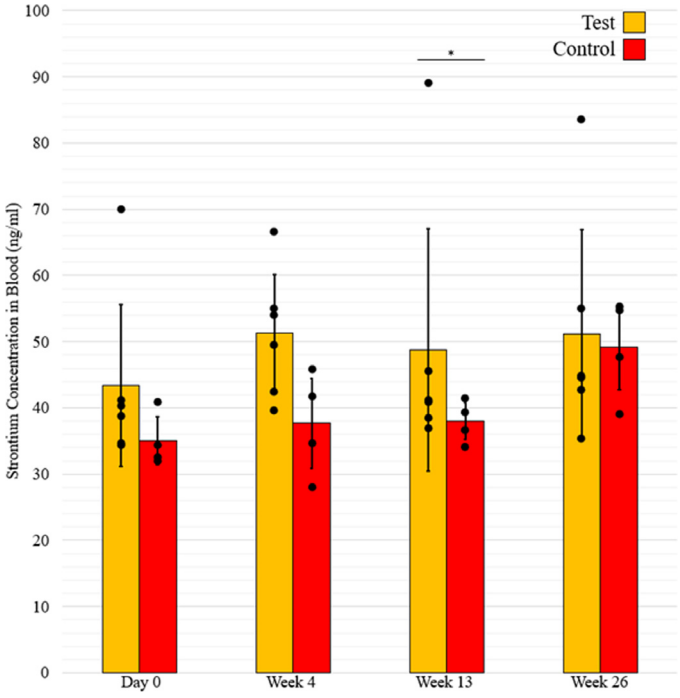

Fig. 6. Strontium Concentration in Blood throughout implantation, $^{*}p<0.05$.

are not statistically significant at $p<0.05$. Comparison of test and control values at each timepoint reveal non-significant fluctuations, except for the week 13 group, which is influenced by the results of one outlier animal in the test group with a naturally higher baseline strontium value – Day 0 samples were taken prior to implantation, and thus represent the non-implanted strontium value for each animal. Globally, standard deviations in the test group were higher than in control group at all time points, due to this test group animal. After exclusion of the results of this sheep from the analysis, standard deviations between control and test groups became comparable, as well as mean concentrations at each time point.

Wool samples were analysed for strontium concentration and the results are shown in Fig. 7. Strontium concentrations in wool were similar between control and test group at Day 0, with all data variations between test and control groups at each timepoint, as well as between baseline (day 0) and each timepoint for both test and control groups, not statistically significant at $p<0.05$. The ap-

Table 3
Biochemistry parameters at implantation and termination. Data expressed as mean ± standard deviation.

Biochemistry Parameter	Units	Test		Control	
		Day 0 (Implantation)	Week 26 (Termination)	Day 0 (Implantation)	Week 26 (Termination)
Sodium	mmol/L	146 ± 2	148 ± 2	145 ± 3	146 ± 2
Potassium (K)	mmol/L	4.8 ± 0.5	4.8 ± 0.2	4.9 ± 0.3	4.7 ± 0.3
Chlorine (Cl)	mmol/L	104 ± 2	107 ± 2	103 ± 2	107 ± 2
Calcium (Ca)	mmol/L	2.54 ± 0.13	2.52 ± 0.09	2.55 ± 0.11	2.44 ± 0.12
Inorganic Phosphorus (P)	mmol/L	1.88 ± 0.45	1.84 ± 0.22	1.72 ± 0.33	1.66 ± 0.39
Blood Urea Nitrogen (BUN)	mmol/L	4.0 ± 1.0	3.2 ± 0.8	4.1 ± 0.6	3.2 ± 1.1
Creatine, serum (CREA)	µmol/L	73 ± 12	73 ± 9	84 ± 10	87 ± 11
Total Cholesterol (CHOL)	mmol/L	1.69 ± 0.39	1.59 ± 0.23	1.71 ± 0.35	1.55 ± 0.22
Triglycerides (TG)	mmol/L	0.20 ± 0.07	0.22 ± 0.06	0.18 ± 0.04	0.22 ± 0.09
Total bilirubin (TBIL)	µmol/L	1.6 ± 0.7	1.5 ± 0.6	1.9 ± 0.6	1.5 ± 1.1
Aspartate Aminotransferase (ASAT)	UI/L	162 ± 50	135 ± 40	147 ± 52	119 ± 27
Alanine Aminotransferase (ALAT)	UI/L	37 ± 8	30 ± 10	33 ± 6	31 ± 6
Alkaline Phosphatase (ALP)	UI/L	87 ± 25	97 ± 45	98 ± 42	106 ± 51

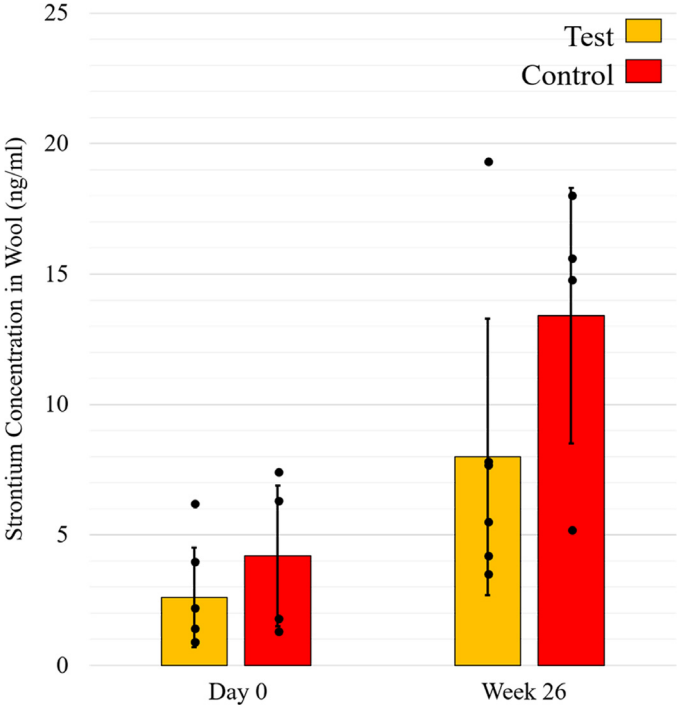

Fig. 7. Strontium Concentration in Wool throughout implantation.

parent increase was more pronounced for the control group compared to the test group and was considered as physiologic or incidental. The presence of test article did not affect strontium concentration in sheep wool.

3.5. Presence of test article does not produce macroscopic or histopathological abnormalities in distant organs

No abnormality assignable to the implanted articles was found in the observed organs. Hard masses in the lungs and livers of the sheep were observed in both groups. These observations were correlated to clinical signs observed during the in-life phase. The histopathological analysis confirmed the diagnosis of expected diseases seen in farm sheep – pneumonia and parasites, and were considered background changes, unrelated to the articles.

There were no treatment-related observations in the distant organs. All macroscopic and histologic findings were incidental and/or associated with background diseases seen in sheep kept in a farm environment. Implant-related histopathologic changes were seen in the spinal nerves in two animals, one from the control group and one from the test group, related to the implantation procedure, not the articles themselves. No muscle lesion was seen in any of the animals.

There was no noticeable difference between the control and test article groups when looking at the lymph nodes (mandibular, axillary, deep caudal cervical lymph nodes). Findings in the lymph nodes of golden-brown granular pigment, mainly within macrophages, were investigated, and determined to be hemosiderin, a degradation product from haemoglobin, not unexpected in lymph nodes as an incidental finding following surgery.

4. Discussion and conclusion

The purpose of this nonclinical GLP study was to evaluate the distant tissue effects and performance of a cervical fusion cage (*Sr-HT-Gahnite Cervical Fusion Cage*) after sheep anterior cervical discectomy and fusion surgery. The test article was compared to a marketed control article (*CTL Amedica Valeo C*) in a sheep cervical spine fusion model 26-week post-implantation. Two cervical sites per sheep were implanted with either the control or the test article with a third article implanted subcutaneously to increase device exposure. The distant tissue effects were evaluated by blood biochemistry analysis and complete blood count, as well as strontium dosage in blood and wool throughout the study, and by qualitative and semi-quantitative histopathologic analysis of selected distant organs.

Regarding the systemic effects, there were no major changes in haematology and clinical chemistry parameters after implantation of the test articles when compared to control group values. Stability of these parameters, including the absence of increased white blood cells demonstrate that the implant was well tolerated throughout implantation, with no observed infection indications. The strontium concentrations in sheep blood and wool were not modified after implantation of the test article, compared to the control group values. Together, the blood and wool results reveal that there is no bolus or sustained systemic distribution of strontium from the device, and no evidence of accumulation of strontium systemically; with comparison to a non-strontium-containing control demonstrating that all fluctuations are attributable to incidental factors. While strontium has been linked to cardiovascular risk factors this effect is thought to be related to the ranelic acid contained in the strontium ranelate medication, rather than strontium itself [26–28]. The results of this study support this, showing that there is no evidence of risk associated with the incorporation of strontium in the Sr-HT-Gahnite fusion cage. It is speculated that any strontium released from device is excreted through normal physiological body processes. In the selected organs that were examined histologically, no test article-related findings were observed.

It is acknowledged that this study is limited by only one terminal timepoint being presented. This is deemed acceptable for systemic safety evaluation, but further studies that investigate the efficacy of the material for clinical use in the spine and other applications should incorporate additional timepoints. Additionally, while this study focused on systemic safety, future quantitative evaluation of acute and local safety at the implantation site is required. The results of this study demonstrate the systemic safety of the Sr-HT-Gahnite Cervical Fusion Cage following ACDF surgery and continued clinical investigation of Sr-HT-Gahnite spinal devices is supported.

Ethics approval

The protocol of this study was approved by the NAMSA Ethical Committee. Each protocol is part of a project authorization that is reviewed every five years by the Ministry of Education, Higher Education and Research, France. The project authorization number associated with this study is #22,062–2,019,091,916,114,859 v4. Animals were managed according to the standard operating procedures of this facility.

Declaration of Competing Interest

The authors declare a potential competing interests through the partial funding of the described studies by the company Allegra Orthopaedics. Study was performed and data generated by test facility, NAMSA.

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