The spinal cord dura mater reaction to nitinol and titanium alloy particles: a 1-year study in rabbits

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Abstract This investigation was undertaken to simulate in an animal model the particles released from a porous nitinol interbody fusion device and to evaluate its consequences on the dura mater, spinal cord and nerve roots, lymph nodes (abdominal para-aortic), and organs (kidneys, spleen, pancreas, liver, and lungs). Our objective was to evaluate the compatibility of the nitinol particles with the dura mater in comparison with titanium alloy. In spite of the great use of metallic devices in spine surgery, the proximity of the spinal cord to the devices raised concerns about the effect of the metal debris that might be released onto the neural tissue. Forty-five New Zealand white female rabbits were divided into three groups: nitinol (treated: $N = 4$ per implantation period), titanium (treated: $N = 4$ per implantation period), and sham rabbits (control: $N = 1$ per observation period). The nitinol and titanium alloy particles were implanted in the spinal canal on the dura mater at the lumbar level L2–L3. The rabbits were sacrificed at 1, 4, 12, 26, and 52 weeks. Histologic sec-

tions from the regional lymph nodes, organs, from remote and implantation sites, were analyzed for any abnormalities and inflammation. Regardless of the implantation time, both nitinol and titanium particles remained at the implantation site and clung to the spinal cord lining soft tissue of the dura mater. The inflammation was limited to the epidural space around the particles and then reduced from acute to mild chronic during the follow-up. The dura mater, subdural space, nerve roots, and the spinal cord were free of reaction. No particles or abnormalities were found either in the lymph nodes or in the organs. In contact with the dura, the nitinol elicits an inflammatory response similar to that of titanium. The tolerance of nitinol by a sensitive tissue such as the dura mater during the span of 1 year of implantation demonstrated the safety of nitinol and its potential use as an intervertebral fusion device.

Keywords Spinal cord · Nitinol · Titanium · Biocompatibility · Intervertebral fusion device

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Introduction

Several cervical and lumbar devices are available to surgically treat degenerative disc diseases. Interbody fusion devices (IFD) and other hardware (plates, rods, and screws) facilitate segmental arthrodesis, while artificial discs permit to preserve segment function. IFD are often referred to as cages, since they present an empty core and external windows that permit bone graft packing to favor fusion. Cages are made from a variety of designs and materials such as titanium-threaded metal, titanium-surgical mesh, and carbon

fiber-reinforced polymer [12, 13, 15, 24–26, 29] in order to support the mechanical stresses that develop before interbody fusion occurs. In spite of their outstanding design, most cages require a bone grafting procedure during surgery. The bone graft is taken from the patient's iliac crest or on the surgery site, and then put into the cage prior to its implantation. However, from the surgeon's point of view this intervention is painful, surgical time is longer, while bone grafting is associated with additional blood loss and potential morbidity [10, 19, 23, 38, 42].

An interesting alternative is the use of a bulk porous material. The biocompatibility and biofunctionality of the porous nitinol IFD, Actipore™ PLFx for the treatment of symptomatic disc degeneration were evaluated in a sheep model for a long period of time [4]. The corrosion resistance [37], as well as the mechanical tests [43, 44], was evaluated successfully.

It has been reported that some cage implants could produce fatigue debris in the instrumented patients [16, 45]. Even though the porous nitinol device was conceived to support the mechanical stresses that develop before the interbody fusion occurs, the concern has been raised regarding the fatigue debris and the reaction of the surrounding tissues, especially the dura mater.

Based on a previous investigation on the dura mater reaction to a polymer material [33], the same animal model and surgical approach were used to investigate the dura mater reaction to the nitinol particles. The purpose of this study was to simulate the unlikely event of debris release from the porous nitinol IFD by means of surgical implantation of nitinol particles in the spinal canal of a rabbit model, and therefore to evaluate the toxicity of the nitinol particles in direct contact with the dura mater and nerve roots. To our knowledge, nitinol or any other metallic material has never been investigated in direct contact with the dura mater. However, in an exhaustive study, Cunningham et al. [18] did investigate the effect of titanium wear debris on spinal arthrodesis in rabbit based on the role of systemic and local cytokines. Histopathological analysis was conducted on the arthrodesis overlying soft tissue, but none on the dura mater. The simulation of the implantation of the particles represents the worst-case scenario regarding the release of the mechanical fatigue debris from an IFD. The rabbits were evaluated for 1, 4, 12, 26, and 52 weeks of implantation following ISO 10993-6 and ASTM standards F763-87 [20, 41]. Medical grade titanium alloy (TiAlV) was used as a comparative material due to its well-known biocompatibility and its current use in intervertebral fusion procedures [24–26]. The histopathological response of both nitinol and TiAlV materials was compared to the

implantation-free sham operation rabbits (control) over dura mater and spinal cord tissue.

Materials and methods

Porous nitinol: mechanical testing and particles generation

The porous nitinol material was produced by self-propagating high temperature synthesis (SHS) according to Itin et al. [22]. An IFD Actipore PLFx was manufactured from porous nitinol material (Biorthex Inc., Montreal, QC, Canada). The porosity of the trapezoidal IFD is $65 \pm 10\%$ with pores of $230 \pm 130 \mu\text{m}$ in diameter (Fig. 1).

In order to evaluate the fatigue and load resistance of the porous nitinol IFD, mechanical tests were performed on the device using a universal testing hydraulic machine MTS, INSTRON 8521 (Instron, Canton, MA, USA). For the fatigue test, Actipore PLFx underwent dynamic axial compressive testing following ASTM 2077-01 standard [9]. Particles were generated at high non-physiological loads ($>2,000 \text{ N}$), with sinusoidal waveforms applied at frequencies of 5 Hz until functional failure after a minimum of 5 M cycles. The deformations, particle loosening, or fissures during testing were periodically documented in order to monitor any diminution in the implant's height. Following ASTM E8 standard [8], a static compression testing was performed on the porous nitinol device. The particles were generated using a testing speed of 0.1 mm/mm/min

Fig. 1 Porous nitinol interbody fusion device Actipore PLFx $230 \pm 130\text{-}\mu\text{m}$ pores, $65 \pm 10\%$ porosity

and force acquisition of 22,000 N representing a high non-physiological application range. The quantity of the particles released from the porous nitinol during the mechanical testing never exceeded 35 mg.

The nitinol particles generated from the fatigue and compression tests were analyzed for size distribution. Most of the particles had a large diameter: 8% <106 μm , 106 μm <10% <150 μm , and 150 μm <82% <300 μm . The size distribution of the particles reflected the worst-case scenario in the unlikely event of porous device failure. The particles resembled small chunks, rather than a powder. For the biocompatibility testing in rabbits, the particles were generated based on the particle size obtained from the mechanical tests. For this purpose, a bar of porous nitinol material was therefore reduced into particles using lathe machining. A total of 10–12 mg of nitinol particles was implanted per animal. Before implantation, the nitinol particles were steam sterilized at 121°C for 30 min.

TiAlV particles

Titanium alloy (TiAlV) particles used as comparative material were commercially obtained from PyroGenesis Inc., Montreal, QC, Canada. The titanium alloy powder represented a mix of various sizes, ranging from 1 to 25 μm in diameter. As in the case of nitinol, the particles were steam sterilized and the same quantity was implanted. However, the ideal TiAlV particles would be those collected from a porous TiAlV device that might be mechanically tested the same way as the nitinol device. Since that kind of device does not exist in the market, the only alternative we had was to test TiAlV commercial particles for comparison with nitinol.

TiAlV powder particles size varied from 1 to 25 μm diameter, the range that covers the sizes that are usually found in case of wear debris of spinal device following unsuccessful fusion [45]. In their investigation, Togawa et al. [45] showed particle generation around titanium mesh cages retrieved from patients following failed fusion or failed fusion with instrumentation failure. The mean period of the device implantation was 27 months (the cages in situ from 2 to 47 months). Particles of debris were seen both intra- and extracellularly. The particles larger than 50 μm were neither phagocytosed nor pinocytosed [27]. However, the size of the particle debris following a spinal failure device remains a controversial topic in terms of particle size and shape. To limit the misinterpretation, the authors assessed wear particles under light microscope by counting only the macrophages that phagocytosed the particles.

Animal model

Forty-five New Zealand white female rabbits (2.5–3 kg; Charles River Laboratories, St-Constant, QC, Canada) were treated in this study. The animals were then grouped according to five different follow-up periods: 1, 4, 12, 26, and 52-week post-implantation periods with either nitinol ($N = 4$ per period), TiAlV ($N = 4$ per period) or sham operation rabbits ($N = 1$ per period). The protocol of the animal study was approved by the ethics committee on Animal Research at the Pediatric Research Center of Sainte-Justine Hospital (Approval L01–23). The guidelines of the Canadian Council on Animal Care (CCAC) for the care and use of experimental animals have also been observed [17]. The guidelines of the International Organization for Standardization [21] and the ASTM standards F981-99 and F763-99 [40, 41] have been followed for implantation and specimen retrieval.

Surgical procedure and particle implantation

Animals were acclimatized for 1 week prior to surgery. Each rabbit was then pre-medicated with a mix of ketamine hydrochloride (30 mg/kg), xylazine (5 mg/kg), and acepromazine (0.5 mg/kg), which was given intramuscularly. After an endotracheal intubation, general anesthesia was maintained with 1.5% halothane and 1.5 l of oxygen (100%) via a respiratory device (Modulflex; Dispo-Med Ltd., Joliette, QC, Canada). The animals were positioned prone with their backs shaved, scrubbed up with proviodine, and sprayed with a mix of iodine/alcohol as an aseptic solution. Eye lubricant was used to avoid conjunctival drying (Lacri-Lube; Allercan Inc., Markham, ON, Canada). All surgeries were performed under strict sterile conditions.

A 4-cm incision was made along the spine at the mid-lumbar region using a scalpel blade number 10. Two spinous processes were exposed and then one spinous process was excised at lumbar level L3. The ligamentum flavum (yellow ligament) was cut with a scalpel blade in order to facilitate access to the spinal canal. The particles were then immediately implanted directly into the spinal canal using a Pasteur pipette with a rubber bulb for the nitinol particles and a curved neck spatula for the TiAlV particles. The implantation site and the well being of the animals were taken into consideration regarding the quantity of the implanted powder (10–12 mg). The sham rabbits underwent the same surgery as the treated rabbits. The incision was then closed in three layers with absorbable sutures: 2–0 Dexon for muscles and 3–0 Dexon for the subcutaneous tissue and skin. The closed wound was coated with

a spray dressing (OpSite; Smith & Nephew Inc., Lachine, QC, Canada). For infection prophylaxis, intravenous injection of antibiotic cefazolin sodium (Kefzol, 125 mg/kg) was performed pre- and post-operatively. A patch of fentanyl transdermal system (each patch contains 2.5 mg, delivery of 25 mcg/h for 72 h) was also given as an analgesic. It was stuck on the rabbit's ear lobe immediately after the pre-medication.

All animals received veterinarian-supervised care under the guidelines established by the Committee on Animal Research at the Pediatric Research Center of Sainte-Justine Hospital. After the surgery, all rabbits were kept in individual cages in order to prevent any excessive movement. They were fed daily with rabbit diet (5079 US Charles River Rodent Animal Diet) and water was distributed *ad libitum*.

Euthanasia: lymph nodes, organs, and spinal cord retrieval

At the end of each follow-up period, all rabbits (treated and sham) were euthanized with an overdose of sodium pentobarbital (Euthanyl 240 mg/ml, 2 ml per 4.5 kg, *i.v.*). In the following order, the regional lymph nodes (abdominal para-aortic), organs (kidneys, spleen, pancreas, liver, and lungs), and the spinal cord were retrieved. The lymph nodes, liver, and spleen are likely to concentrate particles due to their normal hematological roles.

The spinal cord of each rabbit was carefully exposed (Fig. 2). The orthopedic cutters were used to resect the vertebral arch of both sides of the segment. The spinal cord was delicately removed by cutting the nerve roots using a narrowed scalpel. All the specimens were then

Fig. 2 Dissection of the rabbit spinal cord 12-week post-surgery, showing the nitinol particles on the dura mater; the particles clung to the soft tissue

examined with the naked eye and the magnifying glass in order to evaluate the general tissue reaction to the particles of nitinol and TiAlV in comparison with the sham spinal cord. Once examined, the lymph nodes, organs, and spinal cord segments were fixed in 10% buffered neutral formalin for a 1-week period before being processed for histology.

Histopathology

4- μ m tissue sections were prepared from the lymph nodes and organs, which were embedded in paraffin. The slides were stained with hematoxylin–phloxin–safron (HPS). The lumbar spinal segment of the treated rabbits was sliced in three 5-mm blocks (implantation site, adjacent caudal, and cranial sides) and embedded in Epon resin. The latter procedure was a suitable technique for the spinal cord tissue and preservation of the metals around the dura mater. The blocks from the surgical sites of all the sham rabbits' lumbar spinal cords were embedded in paraffin. The treated spinal cords embedded in a resin were prepared using the Exakt-cutting-grinding system (CTBR Ltd., Senneville, QC, Canada). The sections' thickness ranged from 33 to 40 μ m. All the treated and sham spinal cords slides were stained with toluidine blue. The slides were examined under light microscopy and pictures were then taken with a digital camera.

Histopathological examinations were performed following ASTM standard F981-99 [39] based on Turner et al. [46]. It involved scoring each of the criteria on a 0–3 scale; the toxicity was scored on a 0–4 scale, based upon the relative prominence of each item (Table 1). From a general observation of the implantation site, a score of “0” was given if one could not detect an abnormal appearance of the tissue surrounding the implant, “ $\pm$ ” indicated a questionable or very mild reaction of the tissue to the implant. A score of 1+, 2+, and 3+ represented increased degrees of tissue involvement surrounding the implant.

Results

Macroscopic findings

Lymph nodes and organs

The lymph nodes and organs from the rabbits treated with either nitinol or TiAlV did not show any apparent abnormality in the size or color in comparison to the sham rabbit specimens, regardless of the implantation time.

Table 1 Scoring system for tissue response evaluation to nitinol and TiAlV particles

Evaluation of cellular elements		Evaluation of necrosis		Evaluation of toxicity	
Number of elements ^a	Score	Degree	Score	Rating	Score
0	0	Not present	0	Non-toxic	0
1–5	0.5	Minimal present	0.5	Very slight toxic reaction	1
6–15	1	Mild degree of involvement	1	Mild toxic reaction	2
16–25	2	Moderate degree of involvement	2	Moderate toxic reaction	3
26 or more	3	Marked degree of involvement	3	Marked toxic reaction	4

^a The scoring of system of 0–3 is based upon the number of elements (inflammatory cell types) in a high-power field (400×) with an average of five fields

Spinal cords

At 1-week post-surgery, both nitinol and TiAlV showed red soft tissue surrounding the particles. At 4 weeks, the redness of the soft tissue started to dissipate. At 12, 26, and 52 weeks post-surgery, the implantation sites showed the particles trapped into a yellowish soft connective tissue. The dura mater showed a normal vascularization both around the particles and at the remote tissue. Neither necrosis nor swelling was observed either at the nitinol or TiAlV implantation sites regardless of the implantation time. The sham dura mater showed no visible inflammatory reaction.

Histologic findings

Lymph nodes and organs

No particles were found in either the lymph nodes or the organs of the treated rabbits. No metallic particles were found in the macrophages of the lymph nodes. The tissue sections showed the same morphology and cellularity as the specimens of the sham rabbits.

Spinal cords

The sham rabbit sections showed a normal morphology and cellularity of the nervous tissue at 1, 4, 12, 26, and 52-week post-surgery. No inflammation was observed anywhere in the sham spinal cord tissue.

At 1-week post-surgery, the rabbits from the nitinol test group showed an inflammatory reaction mainly limited to the soft tissue at the implantation site. The reaction included residual foci of hemorrhage and adipocyte necrosis, many macrophages with hemosiderin, and some granulocytes, lymphocytes, and plasma cells. Some inflammatory cells extended to the epidural space adjacent to the implantation site; it also involved the cranial and caudal sides. The inflammatory cells did not go through the dura mater on any slide. The

sub-dural space, nerve root, and spinal cord were free of reaction. The TiAlV particles showed the same type of reaction (Fig. 3a, b).

At 4 weeks post-surgery, the rabbits implanted with nitinol particles showed an oedematous and slightly fibrotic tissue with a mild chronic non-specific inflammatory reaction limited to the soft tissue at the implantation site. The inflammatory cells consisted mainly of lymphocytes, plasma cells, and macrophages. Granulocytes were rarely seen. The inflammatory process was more localized without any involvement of

Fig. 3 Spinal cord sections from the implantation sites 1-week post-surgery (a) nitinol particles (b) TiAlV particles. For both materials, the acute inflammation was limited to the soft tissue around the particles in the epidural space, ×25

cranial and caudal sections. The epidural space was almost free of inflammatory cells. Similar to the 1-week results, the dura mater, sub-dural space, nerve root, and spinal cord were also free of reaction. The rabbits treated with TiAlV showed a comparable reaction as well.

At 12 weeks post-surgery, the rabbits from the nitinol test group showed a compact fibrotic tissue with minimal non-specific inflammatory reaction limited to the implantation site of nitinol particles. The inflammatory cells such as lymphocytes, plasma cells, and macrophages were observed. The epidural space was free of inflammatory cells. The sub-dural space, nerve root, and spinal cord were free of reaction. Rabbits implanted with TiAlV particles showed an inflammatory reaction almost identical to the nitinol test group.

At 26 weeks post-surgery, both materials were surrounded by a loose and dense connective tissue. The chronic inflammation was minimal to mild and consisted mainly of macrophages limited to the particles/fibrosis interface (Fig. 4a, b).

At 52 weeks post-surgery, both materials were surrounded more by a dense and well-organized connective

Fig. 4 Spinal cord sections from the implantation sites 26-week post-surgery (a) nitinol particles (b) TiAlV particles. Both materials were surrounded by a loose and dense connective tissue. The macrophages were limited to the interface, $\times 100$

Fig. 5 Spinal cord sections from the implantation sites 52 weeks post-surgery (a) nitinol particles (b) TiAlV particles. Both materials were surrounded by a dense and organized connective tissue. The macrophages were limited to the interface, $\times 100$

tive tissue. The chronic inflammation resembled the 26-week period; it was also limited to the particles/fibrosis interface (Fig. 5a, b). Regardless of follow-up period, there was no infiltration at all in the neurological tissue (spinal cord and nerve roots).

The biological response of the dura mater to both nitinol and TiAlV materials in comparison with implantation-free sham spinal cord is summarized in Table 2, following the scoring system in Table 1.

Discussion

In spinal surgery, with either pedicle screw instrumentation or IFD, the success of the treatment relies on the biocompatibility of the material and on a solid fusion. Some studies had reported the effect of pseudarthrosis (unsuccessful fusion) and the generation of the wear or fatigue debris on the lifespan of the spinal implant [16, 28, 45, 47]. In their investigation, Wang et al. [47] raised their concern regarding the effects of the debris on the surrounding tissues such as the fusion mass, spinal tissues, and neural elements. Regarding

Table 2 Histological evaluation of rabbit spinal cord tissue adjacent to nitinol and TiA1V particles in comparison with spinal cord tissue of sham rabbits at selected time intervals

	Tissue and cell reactions to nitinol and titanium alloy particles									
	1 week		4 weeks		12 weeks		26 weeks		52 weeks	
	Nitinol	TiA1V	Nitinol	TiA1V	Nitinol	TiA1V	Nitinol	TiA1V	Nitinol	TiA1V
Gross response	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Granulocytes	2	2	0.5	0.5	0.5	0.5	0	0	0	0
Lymphocytes	2	2	1	1	0.5	0.5	0.5	0.5	0.5–1	0.5–1
Plasma cells	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5
Macrophages	1	1	1	1	1	1	0.5–1	0.5–1	0.5–1	0.5–1
Giant cells	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fibrosis	None	None	Loose	Loose	Compact	Compact	Compact	Compact	Compact	Compact
Fatty infiltration	None	None	None	None	None	Minimal	Minimal	Minimal	None	None
Dura matter involvement	0	0	0	0	0	0	0	0	0	0
Nuroglial toxicity	0	0	0	0	0	0	0	0	0	0

The sham rabbits did not show any inflammation regardless of time

the nervous tissue reaction, the present investigation may ease some apprehensions on the effects of the metallic debris on the spinal cord in case of implant failure and potential debris release, at least for the first year of the device implantation.

Biocompatibility and good corrosion resistance have been proven with porous nitinol [1–7, 11, 30–32, 35, 36, 39, 48]. Nevertheless, following the surgical procedure and implantation of a previous study on the rabbit model [33], the cytotoxicity of the nitinol particle debris in contact with the spinal cord dura mater was evaluated. The quantity of the nitinol particles tested in the lumbar site represented the worst-case scenario in case of implant-failure. Following intervertebral fusion, the implant will share the load with the newly osseointegrated bone. Since the implant is expected to blend with the adjacent bone in a successful fusion, we assume that any debris that might occur will be trapped in the fibrous and bone tissue, and is unlikely to reach the nerve tissue structures. The reaction of the rabbit's spinal cord to nitinol and titanium was evaluated for 1, 4, 12, 26, and 52 weeks in comparison to the sham rabbits used as the control.

Macroscopically, the particles of both materials (nitinol and titanium) remained at the lumbar implantation site. They were trapped on the dura mater into a loose connective tissue showing an obvious inflammatory reaction at 1-week post-surgery, which was limited to the implantation site without any necrosis or swelling. The nerve roots also seemed normal. The inflammation dissipated at 4 and 12-weeks post-surgery. At 12, 26, and 52 weeks, the mass of particles was embedded with a lattice of yellowish loose connective tissue. The dura mater vascularization and the spinal cord appearance near the implantation

sites resembled the spinal cord dura mater of the sham rabbits used as control.

The histologic analysis showed that regardless of various follow-up periods, the implantation sites of both materials did not reveal any cytotoxicity or necrosis of the dura mater.

At 1-week post-surgery, both materials showed an acute inflammation limited to the epidural space at the implantation site. The cell infiltration resorbed at 4 and 12 weeks. At long term, 26 and 52 weeks, a dense connective tissue embedded the particles. A mild chronic inflammation consisting mainly of macrophages was observed at the particles/fibrosis interface. Regardless of observation periods, the inflammatory cells did not penetrate the dura mater. The sub-dural space, nerve roots, and the spinal cord tissue were free of inflammatory reaction. The morphology and cellularity of the treated spinal cord tissue resembled the sham rabbit spinal cord.

Most of the time, pseudarthrosis is the culprit of the wear or fatigue debris generated around an intervertebral fusion device. The presence of the metallic debris at the metal/tissue interface is responsible for the chronic inflammation, which might lead either to a surgical revision or the failure of the device [16, 45]. It is well known that the success of any intervertebral device relies on a solid fusion. Therefore, in case of a device failure, this might generate particle debris in close vicinity to the dura mater tissue; this investigation showed that the loose connective tissue in the epidural space lining the spinal cord seemed to behave as a shield toward the foreign bodies. The particles triggered a normal inflammatory reaction that was limited to the epidural space surrounding only the implantation site. The biological response to the particles in the

hard or soft spinal tissues can be influenced by the particle size and dose. In a rabbit model, we investigated the worst-case scenario in case of intervertebral device failure. The heterogeneous particle size and shape distribution reflects a particle population that represents the clinical situation.

Regarding the regional lymph nodes and the organs (kidneys, spleen, pancreas, liver, and lungs), no particles were found in any tissue of both the treated groups (nitinol and titanium). The lymph nodes from the treated rabbits showed a normal cell density and structure similar to that of the sham operation specimens. No abnormalities were encountered with the organs, even those with hematological filtering roles (liver and spleen). Since the percentage of larger particles was predominant (92%), the results pertaining to the lack of noticeable particles in the regional lymph nodes were no surprise. The particles of both materials remained on the site embedded in an organized neo-connective tissue.

However, despite the satisfying histological outcome of this study, we were aware of the metallic ion release that might occur after various periods of implantation. From our previous experience with metallic device implantation of titanium alloy and stainless steel screws in sheep lumbar spine, with a follow-up period up to 14 months, we found no systemic metal release in the sheep blood [34]. The analysis of the release of systemic chemical elements such as Al, Cr, Co, Fe, Mo, Ni, Ti, and V in sheep blood was performed by means of flame atomic absorption spectrophotometry (AAS). At the 14 month follow-up, the metallic ion concentrations from sheep implanted with Ti6Al4V resembled those of the control sheep that underwent a sham surgery and that were kept in the same environment as the implanted animals. The metallic concentrations were expressed in ppm (parts per million) and at 14 months the titanium ion in blood was 2–3 ppm.

The investigation of the metal ion release was dismissed in the current study due to the follow-up time, which was only up to 1 year. Based on our former study, it takes more than a one-year follow-up to find any metal release in the blood or target tissues, which depended on the kind of implantation and material.

On the other hand, there is an exhaustive and well-analyzed study by Brayda-Bruno et al [14] regarding the systemic metal diffusion after metallic spinal system implantation in sheep. They tested spinal systems made of Ti alloy and stainless steel, with a follow-up period up to 36 months. The body fluids and target organ tissues (liver, lung, kidney, brain, spleen, and lumbo-aortic lymph nodes) were analyzed by means of

flameless AAS. At 12 months, no metals were found in the fluids or target tissues with either titanium alloy or stainless steel, which corroborates our findings of our former study in sheep. After 12 months of implantation, this investigation corroborates as well the normal histological results of the target organ tissues of the current study in rabbits.

According to Brayda-Bruno et al., after 36 months follow-up, a systemic diffusion of Ti was observed in all tissues of both instrumented sheep with Ti6Al4V (2–16.5 ng/g), except for body fluids and hair.

In any in vivo animal investigation, regardless of the material and the implantation site, the follow-up time is, however, the cornerstone for the host reaction to a foreign material or device.

This investigation of metallic particles on the dura mater and spinal cord in rabbits showed that within the 1-year follow-up, the particles remained in the epidural space with no migration or any side effect on the animal. Based on the mechanical testing in the generation of nitinol particles that might occur in case of pseudarthrosis (non-fusion) of IFD; we simulated the wear debris release from a non-fused IFD in the epidural space and on the dura mater in rabbits.

This investigation showed the worst-case scenario in terms of IFD failure and the amount of different sizes of particles that might occur in vivo. Therefore, during the 1-year follow-up of titanium and nitinol testing, in spite of the particle size that remained at the implantation site, the host inflammatory reaction was similar toward both materials.

In terms of a clinical use, after 1-year follow-up, in case of pseudarthrosis and the likely event of wear debris generation, this simulated study on the rabbit's spinal cord dura mater showed that the particles remain in the epidural space surrounded with mild-chronic inflammation with no harm to the spinal cord tissue.

Conclusion

These investigations demonstrate that nitinol did trigger an inflammatory response similar to the one of Ti alloy after 1-year implantation on spinal cord dura mater of rabbits. For both materials, the inflammation was limited to the epidural space. The dura mater acted as a protective barrier of neural tissue from the inflammatory aspect, which passed from being acute to mildly chronic. However, the success of any IFD is a solid fusion. This outcome depends on the biocompatibility of the material and the design of the implant.

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