ABSTRACT

Introduction

The objective is to assess tracheal reactivity after the deployment of different self-expandable metallic stents (SEMS).

Material and methods

40 New Zealand female rabbits were divided into four groups. Three groups tested SEMS: steel (ST), nitinol (NiTi), and nitinol drug eluting stent (DES). The fourth group was the control group (no stent).

Stents were deployed percutaneously under fluoroscopic control. Animals were assessed by multi-slice, computed tomography (CT) scans, and tracheas were collected for anatomopathological (AP) study. Data from CT and AP were statistically analyzed and correlated.

Results

DES group had the longest stenosis (20.51±14.08 mm vs 5.84±12.43 and 6.57±6.54 mm in NiTi and ST, day 30; p<0.05), and the higher granuloma formation at CT study (50% of
cases). NiTi group showed the lowest degree of stenosis (2.86±6.91% vs 11.28±13.98 and 15.54±25.95% in DES and ST; p<0.05).

In AP, ST group developed an intense proliferative reactivity compared to the other groups. In DES, there was a destructive response in 70% of the animals, while NiTi was the less reactive stent.

CT was superior in detecting wall thickening (positive correlation of 68.9%; p<0.001) than granuloma (NS).

Conclusions

The ST group developed granulomas and significant stenosis. NiTi was the least reactive, and DES caused significant lesions that may be related to drug dosage. Therefore, this type of DES stents is not recommended for the treatment of tracheobronchial stenosis.

KEYWORDS:

stents, drug-eluting stents, tracheal stenosis, airway obstruction, animal experimentation.

MAIN TEXT

INTRODUCTION

Even though surgical exeresis is the primary procedure for the treatment of tracheobronchial stenosis 1, tracheal stenting provides satisfactory management of central airway obstruction in non-surgical patients, along with other minimally invasive techniques, such as radiofrequency, laser ablation, and cryotherapy 2. Silicone prostheses are the most commonly used, but these require the use of a rigid bronchoscope with general anesthesia for implantation. In contrast, metallic stents can be deployed by fluoroscopy or through a flexible
fibrobroncoscope or endotracheal tube under light sedation, providing immediate symptomatic relief. Other advantages of metallic over silicone stents are their higher radial force, better internal to external diameter ratio, less incidence of migration, and decreased risk of mucus obstruction and bacterial colonization. However, these important advantages have been clouded by their long-term complications (such as restenosis due to granuloma formation or tumor growth), and the difficulty of their removal. These issues caused the Food and Drug Administration (FDA) in 2005 to advise against the use of metallic stents in benign lesions.

Nevertheless, since 2005, studies have reported that metallic stents are safe for the treatment of benign and malignant tracheobronchial stenosis. Restenosis, due to intraluminal overgrowth, has mainly been described for steel stents. Studies with laser cut, self-expandable nitinol stents have had better results. However, these nitinol stents are mostly used for other territories such as cardiovascular and biliary tree indications. Antiproliferative drugs are used in cardiovascular indications to avoid restenosis, but experience on the airway is limited.

We hypothesized that DES may merge the advantages of the metallic stents in tracheal stenosis management, while avoiding or attenuating restenosis from intraluminal overgrowth through the mesh and over the ends. Therefore, the purpose of this study was to assess the tracheal responses to three different SEMS (drug-eluting nitinol, nitinol and stainless steel bare stents) in an animal model.

**METHODS**

**Animals and stents**
Forty New Zealand, adult female rabbits (3.95 ± 0.48 kg) were used in this study. The care and use of animals complied with the European Communities Council Directive (86/609/EEC) and the local animal welfare laws, guidelines and policies, and was approved by the ethical committee of the Universidad de Zaragoza. They were randomly distributed into four groups: ST (n=10), steel stent (Wallstent™, Boston Scientific, Natick, MA. USA); NiTi (n=10), nitinol stent (Zilver®Flex™ Vascular Stent,Cook Medical, Bjaeverskov, Denmark); DES (n=10), paclitaxel-eluting nitinol stent (Zilver®PTX® Drug Eluting Peripheral Stent, Cook Medical, Bjaeverskov, Denmark) and control (n=10), with no stent. Both nitinol stents are laser-cut and share exactly the same pattern design, whereas the steel stent is braided wire. All stents were self-expandable and measured 8x40 mm, at a 1:1 ratio to the trachea of the animal model. The stents were deployed percutaneously under general anesthesia and fluoroscopic guidance. Animals were followed for 90 days.

**Stenting technique**

Before stent implantation, all rabbits were checked for sanitary status and fasted for 8 h. They were medicated intramuscularly with 0.5 mg/kg medetomidine (Sedator®, Eurovet Animal Health, The Netherlands) and 25 mg/kg ketamine (Imalgene 1000®, Merial, Barcelona, Spain). Anaesthesia was maintained with 1–2% isofluorane (Isovet, Braun, Barcelona, Spain) by inhalation. Animals were monitored throughout the procedure (Samurai anaesthetic equipment, La Bouvet, Madrid, Spain and Dash 3000 monitor, General Electric Company, Helsinki, Finland).

After positioning animals in supine recumbency and neck hyperextension, and administering 50 mg/kg of oxitetraccline (Terramicina LA, Pfizer, Madrid, Spain), a 5F centimeter-sizing, straight catheter (Aurous®, Cook Medical. Bjaeverskov, Denmark) was introduced into the esophagus to get a measure reference. Tracheal access was gained by puncturing between the
two most cranial tracheal rings using an 18 G catheter-over-needle (Introcan®, Braun, Germany), then 0.15 ml lidocaine (Braun, Barcelona, Spain), was introduced into the trachea. A 0.035 inch hydrophilic guide wire (Radifocus® Guide Wire M Standard type, Terumo, Leuven, Belgium), soaked in lidocaine, was advanced into the trachea, and the catheter was removed. The stent delivery system was inserted directly over the guide wire and placed at a defined position (distal mark of the stent, 1 cm cranial to the tracheal carina) where the stent was deployed under fluoroscopic control (C-arm system BV Endura, Philips, Eindhoven, The Netherlands). Once placed, the delivery system, the guide wire, and the centimeter catheter were removed, and the animal was supervised until recovery (Fig.1).

All procedures were performed equally for the control group, using a delivery stent system without a stent.

**Follow-up and CT study**

After stent placement, all animals were observed for any sign of respiratory tract obstruction and surgical wound infection. The general health status of the animals was assessed by daily clinical examinations.

In cases of death before the end of the study, samples were obtained and processed immediately as per protocol. The only test that was not possible to do in case of death was CT scanning.

CT tracheal studies were performed under sedation 30 and 90 days after stent deployment (Phillips Brilliance CT 16-slice, The Netherlands). CT scans were performed with 1 mm slice thickness, 0.5 mm inter-slice gap, pitch 0.69, and three dimensional reconstructions were obtained.
Parameters assessed in the CT study were: maximum tracheal lumen stenosis in an axial view (%), stenosis length in a sagittal view (mm), and detection of image compatible with granulomas (present or absent).

A stenosis score was also calculated, as described in Table 1, to better evaluate the entire trachea. Five sections in each trachea were defined to simplify CT interpretation: P, tissue immediately cranial to the proximal end of the stent; 0, proximal end of the stent; 2, central part of the stent; 4, distal end of the stent; and D, tissue immediately caudal to the distal end of the stent.

CT studies were blindly reviewed by two senior radiologists who scored each case independently. In cases of disagreement, a third observer was consulted.

**AP study**

Animals were sacrificed at day 90 by intravenous sodium pentobarbital injection (Dolethal®, Vétoquinol, France), and the trachea was sampled for AP studies.

The complete trachea was fixed in 10% formaldehyde. The part containing the metallic stent was embedded in methyl methacrylate, cut by a diamond band saw microtome (EXAKT 310 CS/CP, Norderstedt, Germany), and polished into 8µm-thick sections (EXAKT 400CS Norderstedt, Germany). The segments immediately cranial and caudal to the stents and the total trachea of the control group were paraffin-embedded, and cut into 3-5 µm-thick sections (Leica Reichert-Jung BIOCUT 2030 Microtome, Wetzlar, Germany). Samples were stained with hematoxylin-eosin and studied in an optical microscope (Nikon Eclipse 80i, Nikon Instruments Europe).

The same five sections (P, 0, 2, 4, D) defined for the CT study were also used for the histological study. Parameters studied in histology were: epithelial and subepithelial...
thickness, modification in respiratory epithelium, squamous metaplasia, granuloma formation, and inflammation and vascularization. Some of them were scored according to Table 1.

**Statistical analysis**

All data processing and statistical analyses were performed using SPSS Statistics 17.0 for Windows. An α error of 0.05 was established. Qualitative variables were expressed as frequencies, while quantitative variables were described as mean ± standard deviation. Qualitative variables were compared using the Likelihood Ratio test.

Before comparison, normality of quantitative variables was tested using the Kolmorov-Smirnov test. If the data were normally distributed, the Student’s t-test for independent samples (two means) or analysis of variance, ANOVA (more than two means), were applied. For non-normal distributions, the Mann-Whitney U test or Kruskal-Wallis tests were applied. In cases of ordinal variables, the Wilcoxon test was used.

Correlations were assessed using Spearman’s correlation coefficient (ρ) and the corresponding coefficients of determination (ρ²).

**RESULTS**

The technical success of implantation was 100%. There were no immediate deaths or major complications due to this procedure.

Although most animals were sacrificed at day 90, eight animals (80%) of the ST group died (mean: 31.4 days post-intervention); 2 animals (20%) died in the NiTi group (mean: 39.5 days); 4 animals (40%) of the DES group died (mean: 14.5 days). All deaths were due to causes related to the stent, such as stenosis and infection.

**CT Study**
Stenosis degree.- The maximum tracheal stenosis (%) and its location at days 30 and 90 are represented in Table 2. Whereas maximum tracheal stenosis was located at the proximal end in the steel group, the central segment was more affected in the nitinol group (Fig. 2). Stenosis scores in each group are summarized in Table 3. The ST and DES groups developed higher, but not statistically significant, scores at both days.

Stenosis length.- At day 30 post-implantation, the DES group presented significantly longer stenosis than the other two groups. In all groups, the stenosis length increased at day 90, but stenosis length in the ST and NiTi groups increased more than in the DES group (Table 2). There were no statistical differences between the groups at day 90, or between days 30 and 90 within the same group.

Granuloma Formation.- All groups appeared to have granulomas, which were located in segments 0 and/or 2 (Fig. 3). Scans from the DES group had the greatest intensities of these suspected granulomas. The percentage of cases in the DES group with an apparent granuloma was statistically significant in segment 2 at day 30 (50%, \( p = 0.010 \)), and in segment 0 at day 90 (50%, \( p = 0.018 \)). There were no statistically significant differences in the other groups.

AP study

All AP scores are summarized in Table 4 and AP images are presented in Figure 4.

ST group.- The gross AP result of the ST group was characterized by a thickening of the tracheal wall and significant formation of new vessels. There was an increase of goblet cells and decrease of ciliated ones. The percentage of cases of squamous metaplasia was statistically higher in this group throughout the trachea \( (p<0.001) \). Granuloma formation, mainly observed at the ends of the stent, was also statistically higher in this group. (44.4% of cases). In fact, this was the only group in which more than one granuloma was observed in the same section (11.1% of cases). Acute and chronic inflammation was also observed in this
group, but only acute inflammation was statistically higher than the other groups \((p<0.001)\). New vessel formation was also statistically higher in the ST group (between 22.2% and 55.6% of cases; \(p<0.001\)), comparing to the other groups.

**NiTi group.**- The nitinol stent caused fewer cases of epithelial and subepithelial thickening, which was mild to moderate in appearance, but not severe. The proportion of goblet and ciliated cells was mostly maintained, and in cases of alteration, the disproportion was mild and primarily located at the ends of the stent. We observed squamous metaplasia in segment 4 of 25% of cases, although it was not statistically significant. Few granulomas were detected (12.5% and 10%, at segments 4 and D, respectively) in this group, and had less inflammation and neovascularization.

**DES group.**- DES caused the destruction of the tracheal structure in 7 animals (70%), and stent struts were observed throughout the tracheal wall, with adherences to the surrounding tissue. The epithelium was destroyed in a significant number of cases (40% at the proximal part, 60% at the distal part and 30% at the parts in contact with the stent). Although the epithelium was not as thick in the DES group as in the ST group, it was thicker than in the NiTi group, and the alteration of the epithelium was statistically greater due to its destruction \((p<0.001)\). Overall, squamous metaplasia was not as significant in the DES groups in the ST group, although it was statistically higher in the central segment (40% of cases, \(p=0.020\)). Granulomas were detected in segment 0 in 10% of the cases and in segments 4 and D in the same proportion as NiTi group. Inflammation was primarily composed of lymphocytes and histiocytes, suggesting a chronic response. Neovascularization was also higher in the DES group compared to the NiTi group, but lower than in the ST group.

**Correlation between the CT and Histological Studies**
A positive correlation was calculated between epithelial thickening, as observed in the microscopic study, and stenosis degree, as detected by CT, at day 90 in 68.9% of cases. However, correlation of granuloma detection between the two techniques was not statistically significant ($p=0.880$).

**CONCLUSIONS**

The treatment of choice for symptomatic lesions of the airway is surgical resection, but sometimes other treatment alternatives are required, with or without placement of silicone stents. For tracheobronchial lesions in which surgery is not possible, stenting is an alternative. The use of self-expandable or balloon-expandable metallic stents has spread to treat obstructive or stenotic diseases throughout the body. With increasing frequency, many doctors are deploying expandable metallic stents for the management of patients with diverse endobronchial disorders $^{13}$.

The self-expandable nitinol stent has shown the best results in both benign and malignant lesions of the tracheobronchial tree $^{14, 15}$. However, there has not been a comparative study or series of sufficient sample size to substantiate these results. Our study compared stents of two different types of metals (steel and nitinol alloy). Due to the favorable results of DES in avoiding restenosis in other areas of the body, a third stent made of nitinol, coated with paclitaxel, was also included.

The rabbit trachea is known as very reactive, therefore, we chose this species to assess tracheal wall responses to metallic stents $^{16}$. Furthermore, percutaneous stenting in this animal model is an easy, quick and effective procedure. The five different segments that were analyzed in each sample were useful to evaluate the entire tracheal reactivity and stenosis location.
Steel stents were first used in the tracheobronchial tree with initially favorable results, but quickly revealed shortcomings\textsuperscript{3,17,18}. In De Gregorio et al. study, three out of four steel stents that were used to treat benign lesions in the trachea had to be removed a year after their deployment\textsuperscript{18}. We have corroborated these findings in our study. In fact, mortality was highest in the steel stent group (80%) due to stenosis and infection. Foreign body response and mucus accumulation increased likelihood of infection. Pneumonia was found at necropsy and \textit{Pasteurella multocida} was isolated as the causal agent because it proliferated due to the poor condition of the airway of the animals because of stent deployment. Tracheal stenosis and granulomas in our CT study were, as expected, more significant in the ST group compared to the NiTi group.

Bare, nitinol self-expanding stents had the best performance in both benign and malignant stenotic lesions of the tracheobronchial tree. Several authors have reported acceptable results with this type of stent\textsuperscript{8,20-22}. Our study shows a low rate of epithelial lesions and granulomas in tracheas treated with bare, nitinol stents, as well.

The main cause of restenosis, post-stenting, is tracheal wall thickening due to granuloma formation\textsuperscript{23,24}. Our initial hypothesis was that a paclitaxel DES would improve the results since epithelial reaction would be reduced. Nevertheless, 50% of DES cases developed granulomas in the central segment of the stent at day 30 ($p=0.010$) and in the segment 0 at day 90 ($p=0.018$) in the CT study. Remarkable epithelial destruction was also observed in this group. These results are comparable to Arellano at al. in vitro study, where these DES provoke a prompt and remarkable cellular destruction in fibroblasts cultures\textsuperscript{25}.

Few studies of DES stents in the treatment of airway stenosis have been performed. One report presented a positive effect of a mitomycin C-eluting, bioabsorbable stent implanted in the trachea of rabbits, in comparison to four other types of stents\textsuperscript{26}. Several authors also have
used paclitaxel-eluting stents in the treatment of patients with emphysema to maintain airway patency \(27-30\). Although the results were initially promising, the improvement was only temporary. Except for the airway bypass stents for emphysema, DES are not clinically indicated for airway stenting and futures studies may focus on this issue as a way to prevent granulomas, reduce stent colonization with bacteria and fungi, etc.

Our comparative study agreed with published results for the steel and bare nitinol stents but the findings that we observed in the DES group were surprisingly negative. DES caused severe alterations in all the layers of the tracheal wall, even affecting the cartilage. In order to explain these findings, we hypothesize that the conditions of a DES differ according to location. The correct drug dosage is also important in order to avoid tissue damage \(31\). In another study, high paclitaxel concentration was associated with a worse response in the biliary duct \(32\). The biliary duct and tracheal wall both have different histological structures compared to vessels, where the use of DES has been most effective. Furthermore, blood flow produces a continuous lavage of drug in the vasculature that is not present in other locations, providing a possible explanation for the paclitaxel overdose in the airway in our study. Moreover, the radial forces exerted by the stents in the airway lumen against the airway wall is important in this model, but the design and size of DES and NiTi are the same so differences are due to the drug.

The main limitation of this study is that it was conducted in laboratory animals. The tracheal anatomy and reactivity of the rabbit is similar, but not identical, to humans. In addition, due to the small size, we used stents commercialized for vascular use, with a fixed drug concentration. Data from day 90 in ST group is probably not representative because of the high mortality before this day (80%).
In conclusion, our study confirms the previously reported effects of steel stents, which caused granulomas and significant stenosis. Nitinol stents were the least reactive of the assessed stents. DES caused significant lesions with destruction in all layers of the trachea, possibly related to drug dosage.

ACKNOWLEDGEMENTS

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The guarantor of this study is Miguel Angel de Gregorio, research lead of the Minimally Invasive Techniques Research Group of the University of Zaragoza.

This study was approved by the University Ethical Committee for animal research, and the procedure was conducted in accordance with the "Principles of Laboratory Animal Care" (86/609/CEE Norm. passed by the Spanish legislation through the RD 1021/2005).

Ignacio de Blas, Associate Professor of the Animal Pathology Department of the University of Zaragoza provided statistical advice for the preparation of this manuscript.

REFERENCES


FIGURE LEGENDS

Figure 1: Fluoroscopically-guided percutaneous stent implantation.

Figure 2: (A) Axial, coronal and sagittal views of stenosis at the segments in contact with the stent in a rabbit from the DES group at day 30. (B) Axial, coronal, and sagittal views of a suspected granuloma, located in segment 0, in a rabbit from the ST group at day 30.
Figure 3: Images of a suspected granuloma. (A) CT image, sagittal view with the appearance of granuloma. (B) Macroscopic view of granuloma. (C) Microscopic view (10X) of granuloma around the struts of the stent. (D) Microscopic view (60X) of a giant cell.
Figure 4: Anatomopathological images of the tracheal responses to the assessed stents. First row, gross anatomy; second row, epithelium thickness; third row, epithelium alterations. Haematoxylin-eosin, 60x.
### TABLES

**Table 1. Scores in CT and AP Studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Parameter</th>
<th>Score</th>
<th>Total score is the sum of the values of the five sections(P,0,2,4,D)</th>
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<tbody>
<tr>
<td>CT</td>
<td>Stenosis percentage</td>
<td>0: 0 %</td>
<td>Minimum value = 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1: 1-20 %</td>
<td>Maximum value = 25</td>
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<tr>
<td></td>
<td></td>
<td>2: 21-40 %</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>3: 41-60 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4: 61-80 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5: &gt;80 %</td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>Epithelium thickening</td>
<td>0: Normal &lt;50 µm</td>
<td>Minimum value = 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1: Mild: 50-100 µm</td>
<td>Maximum value = 15</td>
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<td></td>
<td>2: Moderate: 100-150 µm</td>
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<tr>
<td></td>
<td></td>
<td>3: Severe: &gt;150 µm</td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>Subepithelial thickening</td>
<td>0: Normal ≤400 µm</td>
<td>Minimum value = 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1: Mild: 400-600 µm,</td>
<td>Maximum value = 15</td>
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<tr>
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<td></td>
<td>2: Moderate: 600-800 µm</td>
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<td></td>
<td></td>
<td>3: Severe: &gt;800 µm</td>
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### Table 2: Stenosis Assessment in the CT Study

<table>
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<th>Group</th>
<th>Day 30</th>
<th>Day 90</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Segment</td>
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<tr>
<td>Control</td>
<td>0.00 ± 0.00</td>
<td>-</td>
</tr>
<tr>
<td>ST</td>
<td>15.54 ± 25.95</td>
<td>P</td>
</tr>
<tr>
<td>NiTi</td>
<td>2.86 ± 6.91</td>
<td>2</td>
</tr>
<tr>
<td>DES</td>
<td>11.28 ± 13.98</td>
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</tr>
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</table>

### Table 3. Tracheal Lumen Stenosis (Scores, maximum = 25)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 30</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td>ST</td>
<td>6</td>
<td>3.00 ± 3.22c</td>
</tr>
<tr>
<td>NiTi</td>
<td>8</td>
<td>0.63 ± 1.06ab</td>
</tr>
</tbody>
</table>

CT: computerized tomography  
AP: Anatomopathological study  
P, 0, 2, 4 D: Tracheal sections assessed (P, tissue immediately cranial to the proximal end of the stent; 0, proximal end of the stent; 2, central part of the stent; 4, distal end of the stent; and D, tissue immediately caudal to the distal end of the stent)

* ANOVA test. Values with the same superscript indicate no statistical differences between them according to Duncan’s test.

ST, steel stent group; NiTi, nitinol stent group; DES, nitinol drug eluting stent group. P, tissue immediately cranial to the proximal end of the stent; 0, proximal end of the stent; 2, central part of the stent.
<table>
<thead>
<tr>
<th></th>
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<th>Mean ± SD</th>
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<th>Max</th>
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<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
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<tr>
<td>ST</td>
<td>10</td>
<td>5.50 ± 1.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>8</td>
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<td>NiTi</td>
<td>10</td>
<td>2.13 ± 2.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>8</td>
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<td>DES</td>
<td>10</td>
<td>3.67 ± 1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
<td>5</td>
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<tr>
<td>p-value*</td>
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<td>&lt;0.001</td>
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Subepithelial thickening (maximum = 15)

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<tr>
<td>Control</td>
<td>10</td>
<td>0.10 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
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<tr>
<td>ST</td>
<td>10</td>
<td>7.56 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>11</td>
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<tr>
<td>NiTi</td>
<td>10</td>
<td>2.38 ± 2.83&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0</td>
<td>7</td>
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<tr>
<td>DES</td>
<td>10</td>
<td>4.00 ± 3.74&lt;sup&gt;c&lt;/sup&gt;</td>
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Granuloma formation (maximum = 10)

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<th>Max</th>
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<tbody>
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<td>Control</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ST</td>
<td>10</td>
<td>1.11 ± 0.78&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2</td>
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<tr>
<td>NiTi</td>
<td>10</td>
<td>0.25 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2</td>
</tr>
<tr>
<td>DES</td>
<td>10</td>
<td>0.30 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>p-value*</td>
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<td>&lt;0.001</td>
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*Kruskal-Wallis test significance,

Values with the same superscript indicate no statistical differences between them according to paired Mann-Whitney U test.