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Inflammatory Cell Response Evaluation After Total Temporomandibular Joint Replacement in an Animal Experiment

by Maurice Y. Mommaerts[1], Louise Cras[2], Denis Verwaltgen[3], Michael P. Ward[4], Nikolas De Meurechy[5]

[1]European Face Centre, Universitair Ziekenhuis Brussel, Belgium

[2]Department of Pathology, Universitair Ziekenhuis Brussel, Belgium

[3]Sydney School of Veterinary Science, Faculty of Science, University of Sydney, Sydney, Australia

[4]Doctoral School of Life Sciences and Medicine, Vrije Universiteit Brussel, Belgium

Corresponding author: Maurice Mommaerts, Face Ahead Surgeons, Van Putte 20a - 2018 Antwerp, Belgium

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Abstract

Introduction: A sheep animal experiment was conducted to study mastication features, wear of tissue and condylar components and local tissue reaction to a novel patient-specific total Temporomandibular Joint Replacement (TMJR). This paper aims to evaluate the inflammatory response in the peri-artificial tissues, as well as the amount of wear debris produced by the implant.

Materials and Methods: Thirteen TMJRs were implanted in Swifter crossbred sheep. Postoperatively, clinical and inflammatory parameters were monitored daily and recorded weekly. After 268 days, the sheep were euthanized, after which the peri-articular soft tissues were collected, embedded in paraffin and hematoxylin-eosin and Picrosirius Red stained. Five 2-piece porous coated titanium alloy implants and all samples and five randomly selected TMJR components were analyzed. The inflammatory cell response was evaluated by a pathologist. Wear debris analysis of the condylar component was performed. A Kruskal-Wallis non-parametric ANOVA was used to assess differences in macrophage and lymphocyte counts between a group of cases (cord-in-5 sheep, 12 tissue samples and 60 grids) and uncoated (cord-in-7 sheep, 14 tissue samples and 70 grids) condyles. The peri-articular tissues obtained from a sham surgery served as a control group (n = 1 sheep, 2 tissues, 10 samples). A qualitative analysis of the amount of wear debris present (Grade 0-10) was performed.

Results: A significant increase in lymphocytes was found in the DLC-coated (p = 0.0159) and uncoated tissue samples (p = 0.011), compared to the control group. A significant increase in macrophages was found in the DLC-coated tissue samples (p = 0.016). A macroscopic inflammatory cell layer around the nose of the animals developed in screws and is neither experimental group, the concentration of macrophages, nor lymphocytes exceeded the threshold value in orthopedic implants.

Conclusion: None of samples that were examined showed any sign of host-reproductive caused by either wear or infection. No adverse local tissue reactions were noted. We conclude that the novel TMJR is safe for use in sheep. Further research is needed to test and expand any adverse reactions based on our results.

Keywords: Animal Research; Arthroplasty; Inflammation; Joint Prostheses; Pathology; Temporomandibular Joint

Introduction

Since total Temporomandibular Joint Replacement (TMJR) was first introduced in modern medicine, many different systems have been developed [1,2]. Whilst the indications for the prosthetic replacement have been described [3], the ideal system is still unknown. Many patients need to meet, several poorly regulated. Often proper in vivo and in vitro testing of TMJR prostheses is lacking [3], with implantation of improper materials leading to significant detrimental peri-implant effects [4,5].The Stryker TMJ Concepts TMJR, for instance, was withdrawn from development and use of TMJR Stryker has also been tested introduction. A 2nd-hick attributing 54%with coating was found to be an unsuitable articulating surfaces and resulted in the accumulation of wear debris and a foreign body reaction [6]. Similarly, the Biomet TMJ prosthesis was withdrawn from the US market in 2012 after the Food and Drug (FDA)[29], have recommended that all TMJ implants undergo preclinical testing in animals [7]. Implementation (FDA) and the American Association of Oral and Maxillofacial Surgeons (AAOMS) recommended the removal of the implant system in any patients with inflammatory symptoms. The latter illustrates the absolute importance of proper TJR evaluation, before human use [8]. Currently, there is no standardized protocol for preclinical testing. Recently, also TMJ replacement system developed by CADdesign (Clermont, Belgium) [10], three focal points were selected: wear evaluation [9], Lateral Pterygoid Muscle (LPM) attachment [10], and tissue response [11]. The aim of this paper is to evaluate the inflammatory cell response in the peri-artificial models for condylectomy, such as the primate, with a TMJ very similar to that of a human, ethical as well as cost and time restraint issues directed to the use of sheep as a model for TMJ replacement research [12]. Sheep are commonly used in orthopedic [13,14] and TMJ research [15]. Sheep have been shown to survive a mean artificial joint [16] using pre-market TMJ implants [17]. Therefore, the data collected in this study will help to make the data, establishing the safety of the TMJR, makes it a very attractive model of TMJ research [14,18]. As such, 268 days of sheep mastication, equals 22 years of masticatory function of humans [19]. Previously published paper evaluated the wear of the condylar component [20] and the mastication features [19,20]. Instead, post-mortem radiological evaluation wear rate of the tissue and condylar components [9]. The present project aims to evaluate the amount of inflammation of the peri-artificial tissues, which can undergo the inflammatory response in TMJR implants [21]. The number 1 implant failure mode in orthopedic implants [22]. In addition, an interpretation of foreign bodies in the peri-articular tissues was made as well.

Materials and Methods

After obtaining approval by the ethical committee at Medicines Clinic (license number LA 1210576 - code of approval EC MeD 2018-095), fourteen ewes (Swifter crossbreed) aged 2-5 years, with an average weight of 77.4 kg (range: 52–86 kg) and without any missing teeth were enrolled in the study. Two sheep were selected for a pilot surgery. The TMJ in the first ewe was surgically approached, opened and then closed, as would be done in all other animals. However, no condylectomy or prosthesis implantation was performed, thus acting as a control group (Sham). The second sheep had the TMJR implanted unilaterally, as a pilot for the study. After the pilot surgeries, the rest of the animals were randomly assigned to receive either optimized and a standard design implant was established, as elaborately described in our previously published research [5,15]. In total, thirteen sheep were implanted with a TMJR system.

Post-operative evaluation

During the first week, all sheep were kept in solitary confinement. Vital parameters (heart rate, respiratory rate, and body temperature) were evaluated daily. During the daily renewal of the cohesgauze, bandaging the surgery site was evaluated for possible signs of infection. A blood sample was taken daily, allowing for a white cell count (leucocytes), during the first post-operative week. An iongram and eyebrow evaluation of inflammatory parameters was performed twice during this week. After one week, all sheep were put together in a large indoor confinement, where they stayed during the remainder of the study. A weekly clinical evaluation was performed, including a wound evaluation and palpation of the lymph nodes. 268 days after implantation, all the animals were euthanized. A final clinical evaluation and blood sampling was performed, prior to euthanasia. Next injected 5 l/mg/kg (Pul M, 5 M-D Pharma, Leuven, Belgium) and 0.1 mg/kg Midazolam (Dormicum, 5 mg/ml, Roche, Basel, Switzerland). Then, the animals were euthanized by the combination of ketamine 4mg/kg/Midazolam 0.1mg/kg and Pentobarbital 200 mg/ml (Euthanimal 20%) and the removal of 2 mg/kg Embutramide, Lai Vet Pharma Bil, Oudenaarde, Netherlands). The product that was administered to achieve euthanasia remains undisclosed per agreement with Medicines Clinic Ethical Committee.

Sample processing and coloring

After euthanasia, all sheep were decapitated, and the skull was cut in half midsagittally. All the bodies and right sides of the skulls were properly disposed of. The left skull was then further trimmed down by systematically removing the musculature, by the anterior half of the mandible and maxilla, the upper half of the orbit and the orbital contents. The remaining tissue was then fixated by immersion in formaldehyde 4% for three months. Once properly fixated, all samples were decalcified in Decalcifier II (Leica) for 3 days as to remove the osseous structures. The soft tissues were then cut with attention as to not contain scar tissue from the implantation surgery. The tissues were infiltrated through an increasing ethanol series and xylene and embedded in paraffin. Sections were cut at 4 µm thickness, using a microtome (Microm HM 340 E, Thermo Scientific, Waltham, MA) and mounted on slides (Leica Surgipath Poly-Prep Slides). Next, tissues were stained with hematoxylin-eosin (solution A: Hematoxylin for nuclei staining, solution B: background staining caused by excessive use of tissue adhesive and to increase cell transparency. Tissues were then rinsed in running tap water for 10 minutes, followed by rinsing with distilled water. Next, tissues were stained with Picrosirius Red (0.1% Direct Red 80 (Sigma-Aldrich, Bornem, Belgium) in saturated picric acid) for 90 minutes. Tissues were then rinsed in 2 baths of acidified water (5 ml Glacial Acetic Acid (Sigma-Aldrich) in 1 l water) for 2 minutes each. Next, tissues were dehydrated in 3 baths of 100% ethanol for 2 minutes each, cleared in 3 baths of xylene for 5 minutes each and finally mounted with Entellan (Merck, Darmstadt, Germany).

Histological analysis

To obtain an unbiased tissue evaluation, like a Lovins Field Finder grid, a 0.25mm² digital grid was projected on five random locations per tissue sample. (Figure 1) The location of the grid projection onto the tissue was randomized by first discussing the view of the sample itself. In case a grid was set either only partially filled out with tissue, or if a projection of the grid was on the edge of the tissue, the location was to be instance the presence of a blood vessel, a new random grid projection was generated. By means of light microscopic evaluation with a manual cell count being performed, a differentiation between acute and chronic inflammatory response was made. Lymphocytes were identified based on cell morphology. To be counted as a lymphocyte, cells had to be mononuclear with a solitary round and dark blue nucleus (no multiple lobes as in macrophages) and have minimal surrounding cytoplasm. Macrophages had to be round to oval in shape with abundant cytoplasm and a round/oval nucleus. While the cytoplasm is usually "foamy", this does not have to be the case and was not used as an exclusion criterium. A quantitative evaluation of foreign bodies within the grids was undertaken using a polarizing microscope.

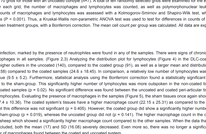

Figure 1: A tissue sample, HE colored, with two 0.25mm² digital grids applied.

Statistical analysis

Two HE- and PSR-stained samples were evaluated per TMJR, resulting in a total of 60 grids analyzed for those sheep who received a DLC-coated condyle (cord-in-5) and 70 grids for those who received uncoated TMJR implants (cord-in-7). Similarly, 10 samples were taken for the control group from sham surgery. For each grid, the number of macrophages and lymphocytes was counted, as well as polymorphonuclear leukocytes if present. Normality of the counts was tested using a Shapiro-Wilk test. Non-normally distributed data was evaluated using a Kruskal-Wallis non-parametric ANOVA test (normal distributions ($P < 0.001$). Thus, a Kruskal-Wallis non-parametric ANOVA test was used to test for differences in counts of macrophages and lymphocytes between groups, with a Bonferroni correction. The mean cell count per group was calculated. All data are expressed as mean ± standard deviation.

Results

No signs of acute infection, marked by the presence of neutrophiles were found in any of the samples. There were signs of chronic inflammation and foreign body reaction, marked by the presence of macrophages and lymphocytes. Figure 2 and Figure 3 show the DLC-coated and uncoated samples, reveals higher outliers in the uncoated (140), compared to the coated group (61), as well as a larger mean and distribution in the uncoated group (34.51 ± 28.58) compared to the coated samples (24.4 ± 14.46). In comparison, a statistically significant increase in the number of lymphocytes was found in the peri-articular sham tissue (5.5 ± 5.2). Furthermore, statistical analysis using the Bonferroni correction found a statistically significant difference in both coated ($p = 0.0159$) and uncoated ($p = 0.011$) tissue samples, compared to the control samples ($p < 0.05$). Similar outputsten in the non-coated tissues ($p = 0.016$), compared to the coated samples ($p < 0.05$). No significant difference was found between the uncoated and coated peri-articular tissue regarding the number of lymphocytes ($p = 0.9$). Similarly, no significant difference was found between the DLC-coated and uncoated tissue samples in the number of macrophages (7.4 ± 10.36). The coated samples showed a significantly higher macrophage count (22.15 ± 25.21) as compared to the uncoated samples (17.76 ± 21.16), yet this difference was not significant ($p = 0.405$). However, the coated group did show a significantly higher number of macrophages compared to the sham-group ($p = 0.016$), whereas the uncoated group did not ($p = 0.141$). No significant macrophage count difference in the sham samples was influenced by one sheep which showed a significantly higher macrophage count compared to the other samples. When the data that was recorded for this sheep was excluded, both the mean (17) and SD (18.38) were significantly decreased. Even more so, there was no longer a significant difference ($p = 0.71$) in the number of macrophages found between the coated and uncoated system.

Figure 2: Peri-articular tissue of a DLC-coated condyle, showing lymphocytes and macrophages present (hematoxylin-eosin stain, original magnification X 100). Green arrow: Lymphocytes. Black arrow: Macrophages.

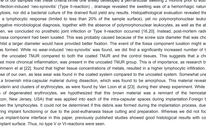

Figure 3: Box plot for macrophage distribution.

Figure 3: Box plot for macrophage distribution.

Discussion

Considering the severe adverse inflammatory reaction that was seen in patients treated with the Vitek-Kent replacement system [1,8,9] , we set out to provide a quantitative analysis of the inflammatory cell types found in sheep treated with a novel TMJR system, developed by CADdelta BV (Ghent, Belgium), as to determine its suitability for human implantation. To properly evaluate periprosthetic tissue reactions, the Synovial-Like Interface Membrane' (SLIM) consensus classification (Table 1) has proven to be an extremely useful system. For a two-synovitis' to be classified as being wear-induced (Type I), more than 25% of the sample had to contain particulate debris. However, in addition, multinucleated foreign-body giant cells should be found as well. In addition to these inflammatory cells, wear particles are present within the macrophages [16-18]. Evaluation of peri-articular tissues of the TMJR in sheep, did not reveal any signs of wear debris, nor multinucleated foreign-body giant cells. Of the 5 samples that were analyzed for this analysis, none of the samples were infiltrated for 25% or more with macrophages. Therefore, none of the samples can be wear-induced neo-synovitis was found in any of our samples. (Table 2, 3) Evaluating the results for the sheep treated with a coated condyle, developing a significantly increased macrophage count compared to the other, uncoated group, could indicate that the coating may not be safe. 10% attempting to find an explanation as to why this one animal showed a more significant microphagous response, compared to the rest of the sheep, a previously performed radiological [21] and wear-analysis [9] was revisited. However, the radiographic images were seen, nor was this found to have had a severely increase amount of volumetric, or linear wear-amount, compared to other sheep.

Type I	Wear-induced synovitis/SLIM
Type II	Infection-induced synovitis/SLIM
Type III	Mixed synovitis/SLIM
Type IV	Indifferent (not wear-induced, not infection-induced) synovitis/SLIM
Type V	Prosthesis-associated arthrofibrosis
Type VI	Adverse local tissue reactions to implant wear particles
Type VII	Local osseous pathologies

Table 1: SLIM consensus classification.

Sheep number	Sample 1
Sheep 1	0.059
Sheep 2	0.021
Sheep 3	0.013
Sheep 4	0.02
Sheep 5	0.059
Sheep 6	0.032

Table 2: Surface area (in mm²) covered per 1mm² with macrophages, in the uncoated tissue samples.

Sheep number	Sample 1
Sheep 1	0.038
Sheep 2	0.052
Sheep 3	0.072
Sheep 4	0.019
Sheep 5	0.042
Sheep 6	0.017

Table 3: Surface area (in mm²) covered per 1mm² with macrophages, in the DLC-coated tissue samples.

In all samples the total wear volume and linear amount of wear that was well acceptable compared to the golden standard and well below the threshold value of 0.1mm/year, as established by Mengelkoch et al. [23]. Similarly, Ward et al. [9] found a linear wear rate of 0.0004mm/year, which averaged at 3.8% of the total linear wear rate in the DLC-coated system and 3.1% in case of the uncoated system. Even with these very respectable results in mind, if the outlier in the DLC-coated samples was removed, this average infiltration rate even dropped further down to 2.9% in addition to this Type I reaction, wear particles can induce a Type II reaction, resulting in adverse local reactions (Type VI-reaction), as was shown in the Vitek-Kent replacement system [8,7]. This Type VI-reaction can be divided into three different groups. Firstly, mainly macrophage pattern with absent or minimal lymphocyte response can be seen [23], a mixed inflammatory pattern, with both macrophages and lymphocytic cells, with variable presence of plasma cells, eosinophils, and mast cells and [5] a granulomatous pattern, predominant or associated with the mixed inflammatory pattern [16,17]. Again, none of our samples showed any of these patterns. We developed a peri-articular sampling 2 months prior to euthanasia, potentially indicative for an infection-induced 'neo-synovitis' (Type II-reaction) , drainage revealed the swelling was of a hemorrhagic nature. A blood sample was taken, revealing a white cell count of 6.5 G/l (range: 4-12 G/l). Therefore, the hemorrhagic swelling was not considered to be an infection-induced hemorrhagic and a hemorrhagic response limited to less than 20% of the sample surface [24] or the presence of polymorphonuclear leukocytes were found. Considering the negative microbiological diagnosis, together with the absence of polymorphonuclear leukocytes, as well as the absence of abscess formation were seen, we concluded no prosthetic joint infection or Type II-reaction was present [16,25]. Instead, post-mortem radiological evaluation revealed that the tissue component had been loaded. This was probably caused because of the screw size diameter that was chosen. This was be that in humans, what a greater diameter would have provided better fixation [26]. However, the current of the tissue component location might well have led to the hematoma that was formed. While no wear-induced 'neo-synovitis' was found, we did find a significantly increased number of lymphocytes in the tissue samples of the uncoated TMJR compared to that of the sham. This is not the correct theory. This suggests that a chronic inflammatory response, or at least more chronical inflammation, was present in the uncoated TMJR group. This is of importance, as research by both Hotca et al. [27] and Ward et al. [28] has shown that DLC-coating can significantly reduce the amount of wear debris that is produced by the TMJR system, which averaged at 3.8% of the total linear wear in case of the DLC-coated system and 3.1% in case of the uncoated system. Even with these very respectable results in mind, if the outlier in the DLC-coated samples was removed, this average infiltration rate even dropped further down to 2.9% In addition to this Type I-reaction, wear particles can induce a Type II-