BIOCOMPATIBILITY ASSESSMENT REPORT

1. Objective

The purpose of this report is to demonstrate, through technical scientific studies, that the materials used in the components of the medical product models in the Exxocut Microdebridement Cannula family have biocompatibility that is recognized as adequate and approved for the uses to which they are put.

2. Evaluations and Discussions

2.1. Model Listing

The products that make up the Exxocut Microdebridement Cannula family are listed in the product's Instructions for Use.
All models, regardless of size/diameter, fitting types or tip types, are manufactured using the following materials:

- Direct Contact
  Cannula kit (inner and outer cannula): AISI 304 stainless steel (ASTM F899)
- No Direct Contact
  Rear coupling: Aluminum and ABS (Acrylonitrile Butadiene Styrene) rear bushing and PTFE (Polytetrafluoroethylene/Teflon) lubricant.

In order to assess biocompatibility requirements, the material used to make the stem and metal tip of the product is taken into account, since it comes into contact with the patient's soft tissue. The stem is made from AISI 304 austenitic stainless steel, in accordance with ASTM F899 (Standard Specification for Wrought Stainless Steels for Surgical Instruments) [1].

2.2. Biocompatibility - AISI 304 stainless steel

The stainless steels used to manufacture surgical and dental instruments have two properties determined by the ASTM F899 standard. The so-called AISI 304 are classified as austenitic (300 series) and this is one of the types of materials defined as one of the most suitable and widely used for the manufacture of surgical instruments.
This type of material is suitable for manufacture and subsequent use as surgical and dental instruments due to a series of characteristics, such as mechanical strength, ease of use for manufacturing processes, but especially resistance to corrosion of alloys with acceptable biocompatibility, among which 304 is one [2].

The materials were tested separately and compared to plastic, which is normally used in vitro studies of the processes involved in cell adhesion, cell proliferation, and the production of GAG, all of which are important components of the ECM.
After all the evaluations carried out and demonstrated in Locci's article [3], we can affirm, according to the results obtained in cell adhesion, proliferation and accumulation of ECM macromolecules, that the 304 and 316 steels show a good level of biocompatibility while, on the contrary, the brazing alloy has some cytotoxic effects on cell functions.

Kocadereli [4] carried out studies to evaluate the release of metal in orthodontic bands, brackets and wires made of stainless steel containing approximately 8-12% nickel and 17-22% chromium.
Chromium and nickel concentrations were measured in the saliva of 45 patients:

a. 15 patients (7 women, 8 men) with an average age of 13.6 years with lower fixed appliances with extraction-free therapy.
b. 15 patients (8 women, 7 men) with an average age of 14.7 years with only upper fixed appliances with extraction-free therapy.
c. The remaining 15 patients (7 women, 8 men), with an average age of 12.8 years, served as controls without orthodontic appliances.

Four stimulated saliva samples were collected from each orthodontic patient at the following times:

a. Before inserting the fixed appliance;
b. 1 week after device insertion;
c. 1 month after device insertion;
d. 2 months after device insertion.

The same 4 saliva samples were collected from each control patient at the same time intervals as for the fixed appliance groups.
During the study, a wide variation was observed in the concentrations of nickel (0.07-3.32 µg / mL) and chromium (0.29-8.0 µg / mL) obtained in the saliva analyses.
No significant differences were found between the samples obtained before and after insertion of the devices. Thus, the study provided no indication that fixed orthodontic appliances affect chromium concentrations in saliva during the first 2 months of treatment. A number of variables such as time of day, diet and salivary flow rate can also affect the composition of saliva.

Haudrechy [5] and Haudrechy [6] carried out studies on the release of nickel from different grades of stainless steel (AISI 303, 304, 304L, 316, 316L, 310S, 430) in artificial sweat.
As a reference for the Ni release limits, the nickel release rate of products intended to come into direct and prolonged contact with the skin was used, restricted in the EU (European Union) to a maximum of 0.5 µg / cm2 / week (limit specified in Directive 94/27 / EEC on nickel - EC 1994).

Under standard test conditions (pH 6.6), nickel release from AISI 303 was only 0.3 µg / ^{cm2} / week, but reducing the pH of the test medium to 4.5 increased releases to 1.4 µg / ^{cm2} / week. Figure 3 shows that for the same conditions, AISI 304 stainless steel released 0.20 µg / ^{cm2} / week, far below what was established.

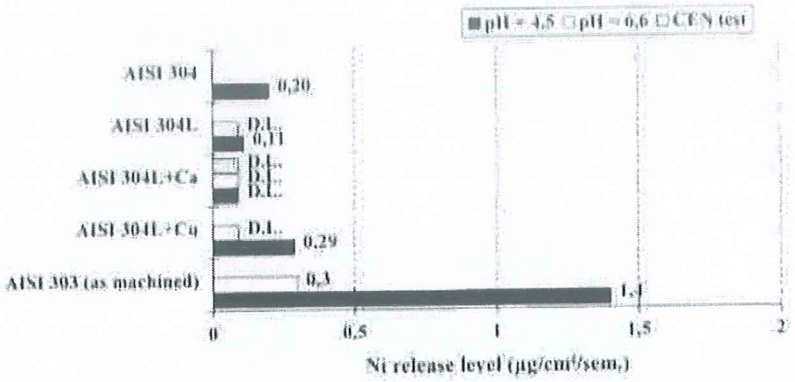

Figure 3 - Ni release for different types of stainless steels in artificial sweat solution (Haudrechy, 1997) [6].

All other types of stainless steel released nickel well below the limit. Lowering the pH to 3.0 or increasing the chloride concentration increased the release of nickel. The release rates of stainless steels were significantly lower than those of pure nickel or nickel-plated steel (100 µg / cm2 / week).
The higher release rates obtained with AISI 303 compared to the other grades are due to its sulphur content, which in combination with manganese can initiate pitting corrosion. Thus, Haudrechy et al. concluded that the use of stainless steels with a high sulphur content in contact with the skin is not recommended. This is also the recommendation of the European stainless steel industry.
Haudrechy et al. also stated that other types of stainless steel can be considered safe when nickel sensitization is considered.

Nie [7] carried out a comparative study of a nanocrystalline 304ss stainless steel made from microcrystalline 304ss plate, which is a commercial and conventional alloy. The chemical composition of both is exactly the same, differing only in their microstructural characteristics.
They were carried out:

a. Ion release behavior in artificial saliva solution.
   The samples were immersed from 1 day to 9 weeks and then their surfaces were analyzed by XPS (X-ray photoelectron spectroscopy).
b. Cytotoxicity test

L-929, NIH3T3, ECV304 and MG63 cell lines were used to evaluate the cytotoxicity of the experimental materials. All types of cell lines were cultured in Dulbecco's modified *Eagle* 's medium (DMEM), 10% fetal bovine serum (FBS), 100ml - 1 penicillin and 100 gml - 1 streptomycin at 37 ◦C in a humidified atmosphere of 5% CO2. The cytotoxicity tests were

carried out by indirect contact. Further details of the methodology can be found in section 2.5 of this article.

With regard to Ni release (figure 4), it can be seen that the rate for the microcrystalline 304ss alloy (commercial) varies between 0.003 and 0.006 ng/ml in the first 7 days of immersion.

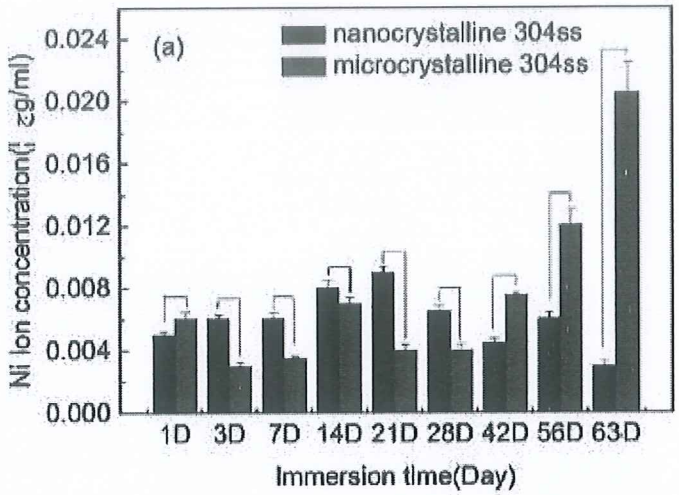

Figure 4 - Release of metallic Ni ions up to 63 days of immersion in artificial saliva and (b) Cr in artificial saliva (figure 4.a - Nie, *et al*, 2011) [7].

A wide variety of human and murine animal cell lines were cultured along with the extractions to assess the cytotoxicity of the specimens given in vitro.

As can be seen in figure 5, for all the proliferation media (NIH3T3, L-929, ECV304 and MG63), there is no significant difference between the experimental materials and the negative control.

With L-929 cell lines. It can be seen that, although there is a slight difference in behavior, the general proliferation of microcrystalline 304ss and nanocrystalline 304ss maintains a high standard of over 80%.

For the other 3 cell lines, the proliferation rate of the 304ss microcrystalline and 304ss nanocrystalline groups remains above 100% up to 4 days of culture, which represents an excellent characteristic of non-toxicity.

In terms of human cell lines, all experimental samples show high-level cell viability above 75% compared to the control at each time point within the 4-day culture.

All in all, there was no noticeable difference in cell viability between nanocrystalline 304ss and microcrystalline 304ss at each fixed time point up to 4 days of culture. This means that both experimental samples maintain an approximately equivalent level of free cytotoxicity in vitro.

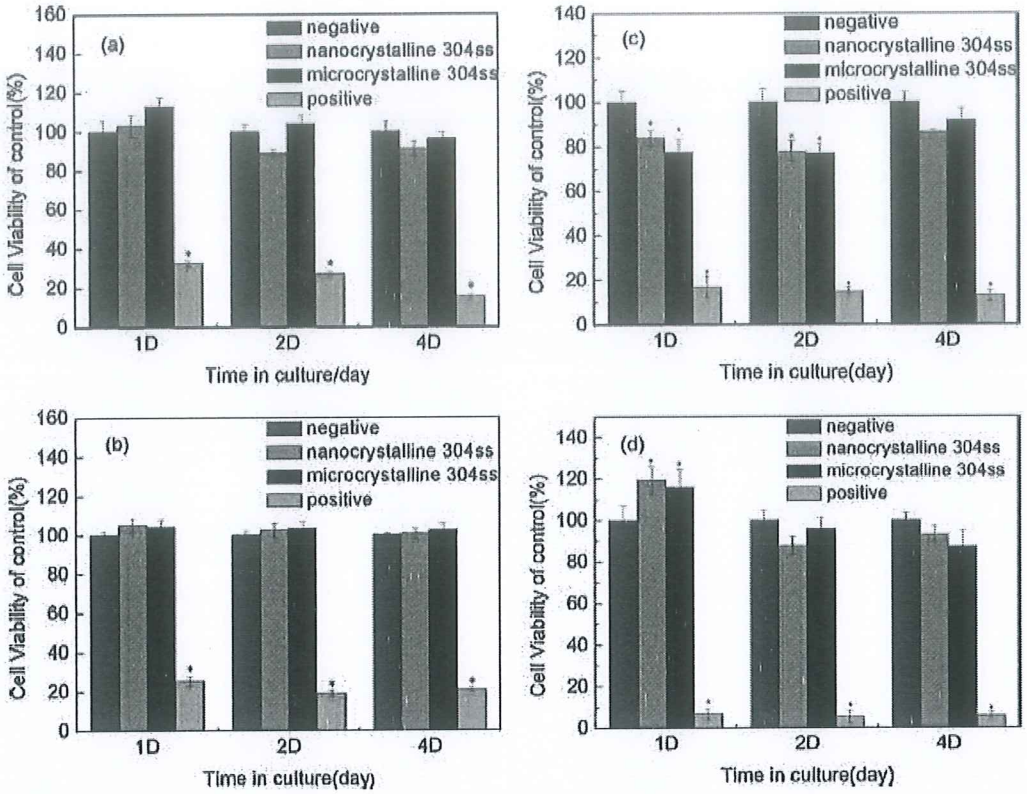

Figure 5 - Cell viability expressed as a percentage of the viability of cells in the control after 1, 2 and 4 days of culture in 304ss microcrystalline and 304ss nanocrystalline extraction media: (a) NIH3T3 cell line; (b) L-929 cell line; (c) ECV304 cell line and (d) MG63 cell line (figure 6 - Nie, *et al*, 2011) [7].

As can be seen in figure 6, Niinomi [8] shows that biocompatibility for 304 stainless steel is very similar to that of 316L stainless steel and CoCr alloy, both implantable metal alloys.

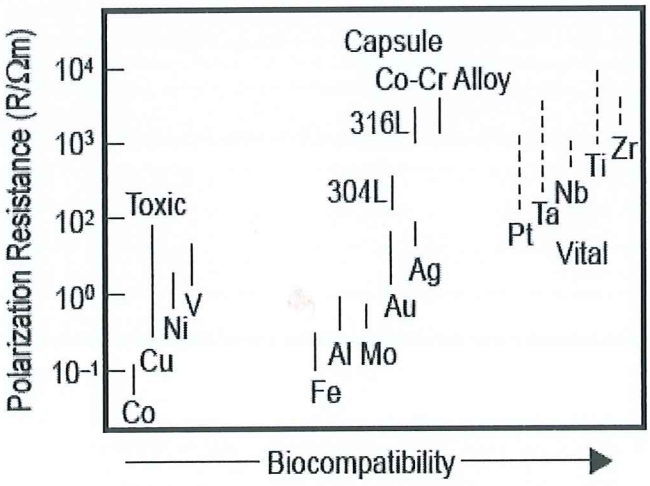

Figure 6 - The biological safety of metals by determining the relationship between polarization resistance and biocompatibility of pure metals, Co-Cr and stainless steels (figure 1.b - Niinomi, 1999) [8].

Taking into account the following aspects and premises:

a. The product's metal rod is a non-implantable component, which can be used for very little time and consequently has very limited contact with any part of the patient's body.
b. The stainless steels used to manufacture the components comply with ASTM F899 specifications.
c. All the studies detailed in section 2 show that AISI 304 stainless steel is biocompatible, and that in short-term use it does not present any restrictions, complications and/or any other type of adverse event that could cause infections or contraindications for the patient, making it safe and effective.
d. It is important to note that all the aspects covered by the articles cited in item 2 refer to medical and/or dental products whose use is not momentary, but either short-term or long-term. Even with these uses, positive and safe aspects have been presented in terms of the biocompatibility of these materials.

3. Conclusions

For all the reasons explained in the articles cited in this report, it is possible to conclude that the materials used to manufacture the components of the products that make up the Exxocut Microdebridement Cannula family, especially the shaft and tip, which come into contact with the patient, are safe, effective and have proven biocompatibility, and are suitable for use without any type of occurrence or adverse event.

4. Bibliographical references

[1] ASTM INTERNATIONAL. **ASTM F899-20(2020)** - Standard Specification for Wrought Stainless Steels for Surgical Instruments. ASTM, 2020.

[2] Fender, William. Brown, Robert. Alloys for Surgical Instruments. **Advanced Materials & Processes**, Volume 163, Issue 4, April 2005 (ASM International).

[3] Locci P, Marinucci L, *et al.* Biocompatibility of alloys used in orthodontics evaluated by cell culture tests. **Journal of Biomedical Materials Research**. 2000 Sep 15;51(4):561-8.

[4] Kocadereli, L., P. A. Atac, *et al.* "Salivary nickel and chromium in patients with fixed orthodontic appliances." **Angle Orthodontist**, Vol 70, No 6, 2000.

[5] Haudrechy, P., J. Foussereau, et al. Nickel release from nickel-plated metals and stainless steels. **Contact Dermatitis**. 31(4): 249-255. 1994.

[6] Haudrechy, P., B. Mantout, *et al.* Nickel release from stainless steels. **Contact Dermatitis**. 37(3): 113-117. 1997.

[7] Nie, F.L., Wangc, S.G. et al. Comparative study on corrosion resistance and in vitro biocompatibility of bulk nanocrystalline and microcrystalline biomedical 304 stainless steel. **Dental Materials**, 27, 677-683, 2011.

[8] Niinomi, Mitsuo. Recent Titanium R&D for Biomedical Applications in Japan. **Journal of the Minerals Metals & Materials Society (JOM)**, Volume 51, Issue 6, pp.32-34. 1999.

According to ASTM F899, table 4, 304 steel is commonly used to manufacture instruments such as: cannula, clamps, forceps, supports, handles, needle vents, retractors, specula, spreaders, suction tubes, tendon passers, among others.
In addition, according to table 5 of the same standard (table 1 below), the material must meet the chemical composition requirements:

Table 1 - Specification for AISI 304 stainless steel, according to table 5 of ASTM F899 [1].

	Elements (%p)							
Type (AISI)	Carbon (C) Max.	Manganese (Mn) Max.	Phosphorus (P) Max.	Sulphur (S) Max.	Silicon (Si) Max.	Chromium (Cr)	Nickel (Ni)	Others
304	0.07	2.00	0.045	0.030	1.00	17.00 - 19.00	8.00 - 11.00	N (0.10 max.)

Nexxmed specifies the same requirements as the ASTM F899 standard through the FES - Raw Material and Component Specification Sheet, FES-MP.AI01, a document belonging to and controlled by the Quality Management System.
All material is inspected upon receipt in accordance with PQ.10.000 - Inspection and Testing.

Locci [3] carried out cytotoxicity tests using 3 types of metal alloys used for orthodontic applications: 304, 316 and 304 stainless steel coated with a brazing alloy.

To carry out the tests, the materials were evaluated using:

a. Cell Culture - Culture obtained from gingival fragments from the area around premolar teeth, washed with Hank's balanced salt solution containing 59 mg/mL of the antibiotic gentamicin. Tissue fragments were transferred to Falcon flasks containing Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum. The cultures were kept in a humidified atmosphere with 5% CO2 at 37°C. Subcultures were obtained 20-30 days later.
b. Cultures on metal substrates - Gingival fibroblasts were collected and seeded at a density of 1 × 106 cells / mL in 9 cm2 wells. After 24 h (subconfluent cultures) or 48 h (confluent cultures) in MEM supplemented with 10% FCS, the discs were transferred to new wells containing 3 mL of MEM and tested under a scanning electron microscope (SEM) for 3H-thymidine incorporation and 3H-glucosamine incorporation as described below.

After subjecting the materials to the cultures, the following evaluations were carried out:

a. Morphological analysis using SEM - Scanning Electron Microscope.
b. Incorporation of 3H-thymidine into DNA
c. Isolation of newly synthesized GAG
d. Determination of protein / metal ions
e. Statistical analysis