

# Evaluation of the Cytotoxicity of AVINENT Dental Implants

## Cytotoxicity Analysis

Once extracts of the material in contact with the culture medium at 24, 48 and 72 hours have been obtained, then a study of their cytotoxicity may be performed. The first step involves treating the cells that are in the incubator.

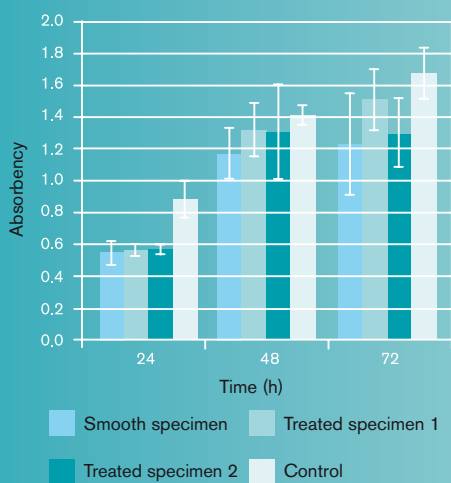
Before beginning the cytotoxicity study, specimens were sterilized and placed on a 40-well plate with 100 µl of culture medium per well, which was incubated for 12 hours.

The technique used for the cytotoxicity study is based on the following process. After 24, 48 and 72 hours of culture, 30 µl of WST, which is a tetrazolium salt, is put into the wells containing 300 µl of culture medium with the cells. Due to the activity of the dehydrogenases of the cell mitochondrion, a link in this molecule becomes broken to give formazan. The combination is left to incubate for one hour to achieve a good reaction level. After this time there is a color change that can be measured by using a PowerWaveX spectrophotometer from Bio-Tek Instruments at a wavelength of 450 nm.

Working parameters are introduced by using the KCJunior program. A larger number of cells causes greater activity by dehydrogenases and therefore a larger concentration of formazan. This product causes an increase in the absorbency of the specimen at the working wavelength (450 nm), and therefore, if the coating is cytotoxic, a clear decrease in absorbency will be seen over time. The death of cells involves lessened activity and less absorbency in the specimen.

## RESULTS

After dilutions have been made to achieve a straight pattern and the cells have been incubated for one hour with the WST-1 reagent, the absorbency reading can then be made. Results of absorbencies for WST tests in direct contact with specimens and for controls are shown in figure 3. These analyses are performed in triplicate to be able to see if any value should be ruled out in the event that there is any disparity in results.



**Figure 2.** Results of the WST test in direct contact with cylinders after 24, 48 and 72 hours (n=3).

## CONCLUSIONS

The coating on the material is not cytotoxic because no cell death is caused. This is seen in the absorbency values found. This reasoning is valid for extracts at 24, 48 and 72 hours.

There has been cell proliferation in both cases, because the cells in contact with the extracts have increased with incubation time.

If a comparison is made between the cell activity results of the cells in contact with the extracts and the results of the negative cytotoxicity control, it may be seen that this proliferation does not go to the same level. This is shown more clearly in the case of 72 hour incubation in figure 3.

When comparing absorbency results between smooth and treated specimens, an improvement in cell proliferation may be seen in the specimens treated with the AVINENT surface. It cannot be concluded that there are any statistically significant differences in cell proliferation between treated specimens 1 and 2.

In summary, from the tests performed, the conclusion may be arrived at that the AVINENT dental implant surface is not cytotoxic and that it improves osteoblastic cell proliferation.

## REFERENCES

[1] Norma española. UNE-EN ISO 10993-5. Evaluación biológica de productos sanitarios Parte 5: Ensayos de citotoxicidad *in vitro*.

[2] *Cell counting and dye exclusion viability assays using a hemacytometer*. Tech Note. Nalge Nunc International Corp. Vol. 3 (25), 2000.