Influence of Electro Discharge Machining of Biodegradable Magnesium on the Biocompatibility

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Abstract

Biodegradable implants are in the focus of recent research approaches in the medical engineering sector for the treatment of many different defects. In comparison to permanent implants the risk of inflammatory reactions is significantly reduced and no foreign material is left in the body using degradable materials. Due to the extraordinary biocompatibility and initial structural stability, similar to the human bone, magnesium alloys are best suited for degradable orthopedic implants. But up until now the degradation of magnesium inside the human body is too fast and therefore the structural stability is lost too early. Newest research suggests that the degradation kinematic as well as the cell response of the implant can be improved by adjusting certain surface properties, e.g. complex micro- and macrostructures. Since these structures are very difficult to be machined with conventional processes, especially for complex and filigree 3D-structures, alternative manufacturing processes need to be developed. Electro Discharge Machining in combination with a Plasma Electrolytic Conversion of the surface is very well suited for the creation of geometries with high aspect ratios and microstructures. The focus of this paper lies on the investigation of the influence of the production processes on the biocompatibility of the machined part. The process chain for such implants will therefore be analyzed in regard to macro and micro surface properties using SEM and EDX-analysis. These results are then compared to biocompatibility testing concerning cell viability and toxicity.

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1. Introduction

The requirements for load bearing permanent orthopedic implants are very high and differentiated. Especially the requirement for a maximum of longevity in a corrosive environment under constantly changing loads is highly demanding for the used biomaterials. Ideally the implant replaces the desired function completely and does not affect the mobility of the patient caused by any discomfort. Implant failures on the other hand are severe and need to be avoided at all costs. Revision surgeries bear risks as every surgery in itself. They typically lead to a more challenging situation for an implantation and may restrict the mobility even further. These surgeries are also accompanied with pain and long recreation times for the patient and the resulting high costs are a serious burden for the health care system. Due to different complications associated with permanent implants the mean lifespan is usually specified between 10 to 15 years. Therefore a high number of revision surgeries is not surprising, especially considering the life expectancy in industrial countries today [1]. An excerpt of typical complications associated with permanent implants leading to an implant failure is listed in table 1. These complications, summarized as insufficient biocompatibility, can be divided into three categories: insufficient mechanical properties, negative biological response of the surrounding tissue, complications arising from the surgery and of course
also a combination of the above mentioned. The functionality and biological compatibility of an implant is influenced by the material itself, e.g. the composition of the used alloy. Another main aspect, if not the most important, is the implant-tissue interface and therefore the surface properties.

| Complication        | Cause                                      |
|---------------------|--------------------------------------------|
| Allergic reaction   | Patient specific intolerance               |
| Inflammatory Response| Release of non-compatible metallic ions or wear debris |
| Implant loosening    | Stress shielding, insufficient bonding between bone and implant |
| Bacterial infection  | Risk of surgery                            |

Surface properties influence the mechanical performance of the implant, either by an insufficient surface integrity, corrosion- or wear resistance. Corrosion and wear particles in turn are a cause of a decrease of the biological compatibility and can lead to negative inflammatory reactions in the surrounding tissue. This is especially the case if foreign materials are accidentally incorporated into the surface. As a result the cell response is also dependent on the implant-tissue interface which can lead to an insufficient osseointegration and ultimately to a loosening of the implant, a typical cause for a revision surgery.

Biodegradable Implants are in the focus of recent research approaches in the medical engineering sector for the treatment of many different defects to overcome the above described drawbacks of permanent implants [2]-[3]. Due to the extraordinary biocompatibility and initial structural stability, similar to the human bone, magnesium alloys are best suited for degradable orthopedic implants. One example is the therapy of missing bone tissue after a tumor extraction or a severe fracture.

In comparison to permanent implants the risk of inflammatory reactions is significantly reduced and no foreign material is left in the body. In this case the degradable magnesium implant will only provide the needed structural stability until it has dissolved completely providing enough time for new bone to grow into the former defect and to recover initial strength. To achieve this goal the degradation must be modified in such a way, that the corrosion rate progresses in the same speed range as the ingrowth of bone occurs. To keep contact with the adjacent bone, the decreasing volume of the implant always has to provide a sufficiently small gap at the implant-tissue interface throughout the whole healing process. But up until now the degradation of magnesium inside the human body is too fast and therefore the structural stability is lost too early. As a result the degradation speed of the implant needs to be decreased and the growth rate of the bone increased. Disregarding the properties of the used biomaterial the implant-tissue interface is the main influencing factor for both aspects leading to similar demands which can be derived from the complications of permanent implants listed in table 1.

Similar to permanent implants the key aspect influencing the corrosion resistance as well as improving the osseointegration is the surface of the implant. The surface properties are influenced by the base material but also to a great extent by the machining technologies representing the footprint of the process chain. Therefore any negative impact on the biological compatibility resulting from the machining process should be avoided. Instead, the implant surface has to be functionalized by properties that improve the therapy. To achieve this goal the different surface properties resulting from the machining process need to be identified and characterized.

Medical research suggests that certain micro and macro structured surfaces can influence the cell response of the surrounding tissue and can therefore improve the growth rate of the recovering bone [4]-[5]. To decrease the corrosion rate of the magnesium implant a change of the surface topography is not sufficient. By using Plasma Electrolytic Conversion (PEC) a barrier layer on the structured magnesium surface can be formed with very high chemical resistance. Therefore a process chain of EDM and PEC is suggested to produce desired surface properties and surface topographies from micro- to macro scale which can be adjusted with great flexibility to meet these demands.

The focus of this paper lies on the investigation on the influence of the production processes on the biocompatibility of the machined part. The process chain for such implants will therefore be analyzed in regard to macro and micro surface properties using SEM and EDX-analysis beginning with magnesium samples machined by EDM. These results are then compared to biocompatibility testing concerning possible toxicities.

2. Electro Discharge Machining

With Electro discharge Machining complex and filigree 3D-microstructures can be machined due to the unique material removal principle leading to very low process forces. As a result implant surfaces can be equipped with various directed surface structures, as seen in fig. 1.
By EDM material is removed due to a thermal impact resulting from a series of high frequent electric discharges between the electrode and the workpiece, which are separated by a dielectric fluid. Therefore a transformation of electrical in thermal energy occurs. As a consequence small volumes are removed by each discharge leaving craters on the surface, leading to the process specific texture of the EDM process. The resulting surface properties are mainly dependent on the amount and duration of thermal energy penetrating into the workpiece during each discharge. Since the thermal energy in turn is dependent on the electric properties of the discharge the surface properties can therefore be adapted by changing the generator parameters. Apart from the generator parameters the thermally influenced surface area is also dependent on the thermal and electrical properties of the machined part as well as the cooling rate of the used dielectric fluid. A consequence of the thermal impact can be a change in residual stress, change in crystalline structure and the creation of pores and cracks, as seen in fig. 2.

![Fig. 1: Microstructures machined by EDM on magnesium surfaces [6]](image)

In case of Wire-EDM all experiments were performed on a Sodick AQ537L machine tool. As wire material a brass wire (CuZn36) was chosen and deionized water as dielectric fluid. The according machining parameters are displayed in table 2.

| Parameter | Description |
|-----------|-------------|
| On | Pulse duration |
| Off | Pulse interval time |
| IP | Maximum discharge current and generator stage |
| MAO | Process control |
| SV | Reference mean voltage |
| V | Open circuit voltage |

A description of the Sodick specific process parameters is given in table 3.

| Parameter | Description |
|-----------|-------------|
| Cut | Wire material |
| Cracks | Cracks |
| Recast layer | Recast layer |
| Redissolution of phases | Redissolution of phases |
| White layer | White layer |

An SEM top view of the machined surface is shown in fig. 3. The performed EDX-analysis of the whole surface only showed magnesium and yttrium which are part of the used magnesium alloy WE43 as well as oxygen resulting from the natural passivation of the surface. The quantitative composition of the workpiece material is displayed in table 4. A more detailed analysis of the surface showed very small particles of copper and zinc. These particles originate with a large probability from the used brass wire.
To prevent even the small amounts of copper deposits, which are categorized as toxic [9], it is common practice to perform finishing cuts with reduced discharge energies to achieve smooth and clean surfaces, as seen in fig. 4. In this case less debris accumulates in the working gap. Apart from the generator parameters the flushing conditions are much better since these cuts are free to the sides.

But since medical engineering research shows that rough surfaces lead to an improved osseointegration alternative process conditions need to be found to keep an increased roughness nevertheless. Therefore the same experiment was redone using a stainless steel wire, which is shown in fig. 5.

Table 4: Composition of WE43 and the stainless steel wire

| WE43         | Stainless steel wire |
|--------------|----------------------|
| Yttrium      | C                    |
| 3.7 - 4.3%   | 0.02%                |
| Rare Earth   | Si                   |
| 2.4 - 4.4%   | 0.37%                |
| Zirconium    | Mn                   |
| 0.4% min     | 0.72%                |
| Magnesium    | P                    |
| balance      | 0.026%               |
|              | S                    |
|              | 0.004%               |
|              | Cr                   |
|              | 18.09%               |
|              | Ni                   |
|              | 10.08%               |

Fig. 3: SEM end EDX-analysis of a machined WE43 surface after rough cut with brass wire and deionized water

The little amount of foreign material is very unusual for a rough cut. A possible explanation is that the working gap is more than double the size compared to the machining of steel resulting in better flushing conditions [8]. In addition to this the observed material removal rate compared to the same steel material is almost 10 times larger. Since the other parameters were constant it can be assumed that the relative tool wear is significantly smaller in comparison.

Fig. 4: SEM and EDX-analysis of the WE43 surface resulting from the 5th trim cut

Table: Composition of WE43 and the stainless steel wire

| WE43         | Stainless steel wire |
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| Zirconium    | Mn                   |
| 0.4% min     | 0.72%                |
| Magnesium    | P                    |
| balance      | 0.026%               |
|              | S                    |
|              | 0.004%               |
|              | Cr                   |
|              | 18.09%               |
|              | Ni                   |
|              | 10.08%               |

Fig. 5: SEM end EDX-analysis of machined WE43 surface after rough cut with stainless steel wire and deionized water

The change in electrode material did not change the general outcome of the experiment. In the overview no foreign material was identified by the performed EDX-analysis. In the detailed view again only very small particles originating from the tool electrode were found and the amount was even smaller in comparison to the base material. But due to the fact that the single parts of the steel alloy, e.g. chromium and nickel, are regarded as toxic as well the resulting biocompatibility should not be improved by the use of this wire. As an alternative directed structures can be machined using trim cuts to increase the overall roughness as shown in fig. 5.

4. Analysis of Magnesium Surfaces Machined by Sinking-EDM

Typical process conditions for macro Sinking-EDM applications are the use of oil-based dielectric fluids and copper or graphite electrodes. For every experiment an
AgieCharmilles Form 2000 machine tool was used. Since graphite is becoming the more preferred choice especially for roughing and since it also has better biocompatibility the tests were limited to graphite as electrode material. The process parameters of the applied roughing technology are shown in table 5.

Table 5: Used process parameters of the AgieCharmilles Form 2000

| Parameters* | Values |
|-------------|--------|
| Discharge current I | 38     |
| Pulse duration T | 133    |
| Ratio of flushing movement Teros | 0.4    |
| Pulse interval time P | 38     |

*Parameters are AgieCharmilles specific and do not necessarily resemble a physical quantity

The according SEM pictures and EDX-analysis is shown in Fig. 6.

5. Toxicity Tests of We43 Surfaces Machined by W-EDM

To evaluate a possible toxicity of surfaces machined by EDM tests were performed following the DIN EN ISO 10993-5 “Biological evaluation of medical products: Verifcation of in-vitro-toxicity” [10]. For the testing procedure the NCTC- clone 929 cell line was used. The cells were cultivated following the according protocol of the norm.

The first test was performed with WE43 samples machined by Wire-EDM using a brass wire since this process condition was identified in the prior analysis as most likely cause of a decrease of the biocompatibility. Three different sets of generator parameters were applied to identify influences arising from roughing and finishing technologies shown in table 2. The first performed toxicity test measures the activity of the cellular enzymes to reduce the water soluble yellow tetrazolium dye, MTT, to its insoluble formazan, which has a purple color. The amount of generated formazan gives an indication of the cell viability and was determined after 6, 24 and 48 hours.

![Fig. 6: SEM and EDX-analysis WE43 surface after roughing with oil based dielectric fluid and graphite electrode](image)

Fig. 6: SEM and EDX-analysis WE43 surface after roughing with oil based dielectric fluid and graphite electrode

Similar to the results of the W-EDM process virtually no foreign material was found on the surface. Nevertheless traces of carbon could still be present on the surface because this light element is not easily detected by an EDX-scan. In addition to this only the surface of the magnesium sample was machined and therefore the flushing conditions were ideal. The results may be different if deep cavities are machined. In this case the flushing conditions are worse and the probability of foreign material deposits is significantly increased. One possible solution could be to exchange the oil-based dielectric with deionized water also for Sinking-EDM.

![Fig. 7: MTT test for cell viability results after 6, 24 and 48 hours](image)

Fig. 7: MTT test for cell viability results after 6, 24 and 48 hours

According to the norm a value below 70% of the control group is considered as toxic. In fig. 7 the results for the MTT test are shown. The three magnesium samples were compared to the control fluid which was set to 100% and to a conventionally machined Ti6Al4V sample. The resulting values where derived from 6 individual tests each. Only the rough cut with a Ra value of 5 µm leads to a cell viability below 70% of the control after 6 hours which complies with the found foreign deposits in the performed SEM analysis. The two other samples which were machined with reduced discharge energies with a resulting Ra value of 0.57 and 2.87 µm achieve better values compared to titanium. As stated before the probability of foreign deposits is
decreased with reduced discharge energies and better flushing conditions.

After 6 hours the viability of the 929-Cells decreased significantly. This probably correlates with the accumulation of gas bubbles and corrosion particles which were observed inside the petri dish of the magnesium test samples. During the corrosion high concentrations of hydrogen gas is released which is probably the cause for the disturbance of the cells. The results after the subsequent PEC process are expected to prevent this negative course.

![Graph showing LDH levels for 6, 24, and 48 hours](image)

Fig. 8: LDH levels for possible tissue damage after 6, 24 and 48 hours

To receive an indication of the actual cell damage the lactate dehydrogenase (LDH) level of the cell culture was measured. This enzyme catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD⁺. Usually the level of LDH is low but in case of tissue damage cells release more LDH into the bloodstream. In this case a lower value indicates a higher biocompatibility. According to the MTT tests the LDH level was measured for all test samples after 6, 24 and 48 hours. After 6 hours the LDH level of the magnesium samples is a little higher compared to the control and also compared to the titanium samples, as shown in fig. 8. In contrast to the MTT test the results of the magnesium samples improve and decrease significantly under the value of the control. The LDH level of the titanium sample on the other hand increases and comes to a similar value. A critical tissue damage arising from the EDM process therefore seems unlikely especially after finishing cuts.

6. Conclusion

In this paper the influence of the EDM process on the biocompatibility of magnesium samples was investigated. For this purpose SEM and EDX-analysis of WE43 surfaces were performed. In case of Wire-EDM rough cuts only very little foreign material originating from the used electrode was detected on the machined surfaces. After Sinking-EDM as well as after finishing with Wire-EDM no foreign material at all could be detected besides the base material. This complies with the results of the in-vitro toxicity testing. Only the rough cut decreased the viability of the used cell line after 6 hours. The progression of the magnesium corrosion however led to a continues decrease of viability in all tested magnesium samples. A tissue damage indicted by an increased LDH level could not be observed in any of the tests.

A certain toxicity of machined magnesium surfaces by EDM was observed which can easily be avoided with optimized process parameters. Only the corrosion of magnesium remains a great issue which needs to be prevented by the following PEC process step.

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