Final report	
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Part I: Text

Abstract in English

The aim of the project "Nano Metal Injection Moulding of Metal Surfaces for the Functionalisation of Metal Surfaces" was to develop an innovative manufacturing process, nano metal powder injection moulding, for the integrated production of functional micro-and nanostructured surfaces.

Implant surface structure and chemistry determine the fate of cells coming in contact with the implants. Surface structuring can be characterised according to the size of the structures (nano, micro) and the type (random, regular defined, shape). Today most current bone related implants undergo special surface treatments applicable to large areas such as etching and sandblasting as an additional step after manufacturing, thus having random structures of nm to µm size.
Several studies report that regular defined structures may have an additional positive effect on cells of the osteoblast lineage and on implant bone contact ratio [1, 2, 3, 4, 5]. For micro-and nanostructuring of metals different production technologies exist (e.g. micro-machining, spark erosion, laser ablation, LIGA technique). However, they have the disadvantage that only a limited range of metals can be processed and that they are generally unsuitable for larger scale productions [6]. In addition, these techniques could only be applied as post processing for medical implants.
The new process developed during this project presents a more economical approach to produce regular patterned surfaces on the bases of the metal injection moulding process (MIM). MIM is a powder metallurgical process designed for large scale production of small, intricate metallic parts. It is widely used in industry as it provides cost-effective net-shape manufacturing of metal parts for a variety of materials [7]. A metal powder of about 20 to 40 µm in particle diameter is mixed with an organic binder (=> feedstock), injection moulded like a polymer, the binder removed and the powder sintered to full density. Growing markets for such metal parts are found in the fields of, e.g., medicine, automobiles, jewellery and sports. Further developments use powders of 5 µm for the production of micro metallic parts and microstructured surfaces [8, 9, 10].
During the last three years, the MIM process was specially modified in order to process hybrid micro/nano powder mixtures, which is why it is also referred to as "Nano-MIM". The scope of the project included the development of the complete processing chain for Nano-MIM: Feedstock formulation, injection moulding, debinding and sintering. The capacity to replicated structures of the mould at micrometer range was evaluated by using mould with regular array of hemisphere-like cavities. The resulting materials with protruding hemispheres of 5, 30 and 50 um in diameter and an interhemisphere distance of 20 µm are termed 5_20, 30_20 and 50_20 and validated by investigating surface topography, microstructure, biocompatibility and cell performance.
Most efforts have been made for the biocompatible stainless steel 316L but also studies with pure titanium have been conducted. As evaluated for stainless steel the use of very fine powders together with new sintering strategies enabled us to successfully replicate regular hemispheric surface patterns with features down to 5 µm in diameter on a very reproducible way. Furthermore, an additional irregular sub-structure, caused by the formation of grain boundaries induced a defined roughness of the samples. This roughness could be reproducibly defined by the percentage of metal nanoparticles added to the binder. Mechanical testing of the materials revealed an extreme high density and tensile strength of the resulting stainless steel material.
Biological evaluation of the materials using primary adult human bone marrow stromal (HBMCs) revealed that the obtained materials were cytocompatible (non-toxic). The topographical micrometer sized structures explored in this study induced considerable changes in cell shape. In case of stainless steel surfaces, a significant increase in cell shape compactness was associated with the presence of micrometer structures. In addition, focal adhesion plaque size of HBMC was increased due to the presence of these structures. Moreover, it could be demonstrated that the seeded cells obtained a unique, three-dimensional, conformation, i.e. the cells appeared to mainly adhere to the protruding hemisphere structures on the surfaces. The hemispheres increased cell mobility as measured after 7 days in culture and affected cell differentiation. The reaction of cell on stainless steel surfaces coated with titanium differed from that of uncoated surfaces as measured by the cell adhesion.

Abstract in German

Das Ziel des Projektes "Nano Metal Injection Moulding von Metalloberflächen für die Funktionalisierung von Metalloberflächen" war die Entwicklung eines innovativen Herstellungsverfahrens, Nano-Metallpulverspritzgießen, für die integrierte Produktion von funktionellen mikro- und nanostrukturierten Oberflächen. Die Struktur und Chemie einer Implantatoberfläche bestimmen das Schicksal der Zellen, die in Kontakt mit den Implantaten kommen. Oberflächenstrukturierung lassen sich nach der Größe der Strukturen (Nano, Mikro) und der Art (zufällig, regelmäßig definiert, Form) charakterisieren. Heute durchlaufen die meisten aktuellen Knochenimplantate eine spezielle Oberflächenbehandlung, anwendbar für große Bereiche, so wird z.B. Ätzen und Sandstrahlen, die in einem weiteren Schritt nach der Herstellung für zufällige Strukturen im Nanometer- bis Mikrometerbereich sorgen. Mehrere Studien berichten, dass regelmäßige definierte Strukturen eine zusätzliche positive Wirkung auf Zellen der Osteoblasten-Linie und auf das Implantat/Knochenkontakt-Verhältnis haben [1, 2, 3, 4, 5]. Für Mikro- und Nanostrukturierung von Metallen existieren unterschiedliche Fertigungstechnologien (z.B. Feinzerspanung, Funkenerodieren, Laserablation, LIGA-Technik). Sie haben jedoch den Nachteil, dass nur eine begrenzte Auswahl von Metallen verarbeitet werden kann und sie in der Regel ungeeignet für größere Produktionen sind [6]. Darüber hinaus können diese Techniken nur als Nachbearbeitungsschritte für medizinische Implantate eingesetzt werden. Das neue Verfahren, welches im Rahmen dieses Projektes entwickelt wurde, stellt einen ökonomischeren Ansatz für die Oberflächenstrukturierung auf den Grundlagen des Metallpulverspritzgießens (MIM) dar. MIM ist ein pulvermetallurgisches Verfahren und wurde für die großtechnische Herstellung von kleinen, komplexen Metallteilen entwickelt. In der Industrie wird das Verfahren verbreitet für die kostengünstige endkonturnahe Fertigung von Metallteilen eingesetzt und bietet den Vorteil eine Vielzahl von Materialien verarbeiten zu können [7]. Ein Metallpulver von etwa 20 bis 40 µm Partikeldurchmesser wird mit einem organischen Binder gemischt (=> Feedstock), wie ein Polymer spritzgegossen, entbindert und anschließend zu vollständiger Dichte gesintert. Wachsende Märkte für solche Metallteile sind z.B. die Bereiche Medizin, Autos, Schmuck und Sport. Weitere Entwicklungen nutzen Pulver von 5 µm für die Herstellung von metallischen Mikroteilen und mikrostrukturierten Oberflächen [8, 9, 10]. In den letzten drei Jahren wurde das MIM-Verfahren speziell modifiziert, um im sogenannten "Nano-MIM"-Prozess hybride Mikro-Nano-Pulvermischungen zu verarbeiten. Die Aufgabe des Projekts war die Entwicklung der gesamten Prozesskette für Nano-MIM: Feedstockformulierung, Spritzgießen, Entbindern und Sintern. Die Prozessfähigkeit für die Abformung von Strukturen im Mikrometerbereich wurde anhand einer Spritzgussform mit regelmäßig angeordneten Halbkugelstrukturen ausgewertet. Die resultierenden Proben mit hervorstehenden Hemisphären von 5, 30 und 50 µm Durchmesser und einem Abstand von jeweils 20 µm werden im Folgenden mit 5_20, 30_20 50_20 bezeichnet und durch Untersuchungen der Oberflächentopographie, Mikrostruktur, Biokompatibilität und Zelleverhalten validiert. Die meisten Anstrengungen wurden hinsichtlich des biokompatiblen Edelstahls 316L gemacht, aber auch Studien mit reinem Titan wurden durchgeführt. Mit dem Edelstahl ließen sich durch die Verwendung sehr feiner Pulver zusammen mit neuen Sinterstrategien erfolgreich regelmäßige Oberflächenstrukturen mit Abmessungen von 5 µm im Durchmesser reproduzierbar abformen. Außerdem wurde eine zusätzliche unregelmäßige Substruktur durch die Bildung von Korngrenzen erzeugt, die eine definierte Rauheit der Proben induziert. Diese Rauheit konnte reproduzierbar durch den zugesetzten Prozentsatz der Nanometallpartikel reguliert werden. Mechanische Prüfung der Materialien ergaben eine extrem hohe Dichte und Festigkeit für den Werkstoff Edelstahl. Die biologische Beurteilung der Materialien mithilfe primärer adulter menschlicher Knochenmarkstammzellen (HBMCs) ergab, dass die Materialien eine gute Zellkompatibilität besitzen (ungiftig). Die topographischen Mikrostrukturen, die in dieser Studie untersucht wurden, induzierten eine erhebliche Veränderung der Zellform. Im Falle der Edelstahloberflächen wurde eine signifikante Zunahme der Kompaktheit der Zellform bei Anwesenheit von Mikrostrukturen beobachtet. Darüber hinaus waren die fokalen Adhäsionsstellen der HBMC auf den Halbschalen erhöht. Zudem konnte gezeigt werden, dass die ausgesäten Zellen eine einzigartige, dreidimensionale Gestalt annehmen, d.h. die Zellen offenbar vor allem an den vorstehenden Hemisphärenstrukturen auf den Oberflächen haften. Die Hemisphären erhöhten außerdem die Zellmobilität, was anhand von Messungen nach 7 Tagen in Kultur und der Zelldifferenzierung gemessen wurde. Die Reaktion der Zelle auf titanbeschichteten Edelstahloberflächen und unbeschichteten Oberflächen unterscheidet sich im Zelladhäsionsverhalten.

Summary of the past 3 years of work

For injection moulding of micro-patterned samples the biocompatible 316L stainless steel was chosen during the first part of the project. To prepare the appropriate feedstock, a method was developed under inert atmosphere, with which it was possible to prepare mouldable feedstocks with nano powder additions up to 33%. Using an iron powder it was possible to shown that the processing of feedstock with 100% nano powder was possible as well. Moulding of specimens was achieved with all the processed feedstock, although appropriate process adjustments (higher injection pressures and moulding temperature) were required depending on the percentage of nano powder additions. The capacity to replicated structures of the mould at micrometer range was evaluated by using mould with regular array of hemisphere-like cavities. The materials were sintered under various process conditions and densities over 98% of the theoretical density have been achieved. The resulting materials with protruding hemispheres of 5, 30 and 50 um in diameter and an interhemisphere distance of 20 µm are termed 5_20, 30_20 and 50_20 and the at the micrometer scale non-structured control NS. The positive influence of nano powders on the formation of sub-structuring was confirmed in the SEM. With a higher proportion of nano powders, the sintering temperature could be reduced and sub-structuring with features down to the sub-micron range were provoked. A first series of samples were tested at Empa regarding biocompatibility (cytocompatibility and bioactivity). The cytocompatibility and the positive impact of micro-patterning on the bioactivity were already confirmed on a material with low nano powder share of 10%.

In the course of the second project year, improved moulding quality of the 316L nano-particle containing feedstock was achieved by using an industrial micro-injection moulding machine (Battenfeld, Microsystem 50). Better process control allowed further optimisation the moulding temperature, pressure and part extraction, leading to higher reproducibility of the micro patterns. Especially, the replication of the previously challenging 5 µm hemisphere array as a result of nano particle incorporation was achieved, which significantly enhances process applicability. Additionally, a sintering program for a reliable production of micro-patterned stainless steel samples has been developed as a standard. The essential relationship between density, surface and bioactivity were determined depending on the material basis of the sintering program. The influence of the nano powder and the sintering conditions on the formation of the desired sub-structure could be confirmed by white light profilometry through specific adjustment of parameters and atomic force microscopy. In parallel, the sintering technique has been extended to new methods, and experiments were carried out with microwave sintering and spark plasma sintering. The results show that the high heating rates achievable hereby suppress grain growth evidently, and so a further refinement of the sub-structure is possible. Furthermore, measurements were continued to characterise the surface topography, which displayed the influence of the nano powder and the sintering conditions on the formation of the desired sub-structure. In parallel with the first studies of titanium as an additional biocompatible material were carried out. Here a modified binder system was used due to the material requirements. First test moulding, sintering and characterisation of titanium were carried out.

To receive information concerning biocompatibility, in a first step it was proven that no toxic components were released from the samples. In a second step human bone marrow cells from adult patients were seeded onto the different materials and their behaviour (adhesion, cell migration) and their properties (cytoskeleton, differentiation) were observed over several days or weeks. Summarising, it was shown that all materials are cytocompatible and bioactive. For example, a significant increase in strength of cell adhesion to the substrates on 50_20 and 30_20 hemisphere surfaces as compared to non-structured (NS) control samples was found. Furthermore a change in cell shape was observed: when in contact with the patterned surfaces cells showed an increase in cell circularity in combination with a unique 3-dimensional conformation. Cell motility was also increased by the presence of both 50_20 and 30_20 structured surfaces as evaluated after 7 days in culture. 24 hours after seeding no difference between the samples in migration velocity were seen.

In the last year of the project at IFAM were made some further investigations on the previously produced stainless steel samples. For example further mechanical studies were carried out. Also, simulations of the filling behaviour, as well as pressure and temperature distribution during the injection process were calculated. These results give now a complete picture of the entire process.

Regarding the development of injection moulded titanium samples, additional binder variations have been conducted and two different injection moulding machines were utilised. Through the installation of a mixer inside a glovebox with permanent inert argon atmosphere, the oxidation of the titanium

powder during the mixing process was avoided completely. This was especially important for the purity of the samples, consequently leading to the successful preparation of highly pure Grade-1 samples. Also grain refinement by adding nano powders was applicable, in analogy to the previous results for stainless steel.
At Empa it was evaluated in how far the presence of hemispheres affected the cell proliferation. It was found that although cell number was reduced on the microstructured surfaces a significant increase in the percentage cells that actively proliferated cells surface microstructure could be observed after 7 days in culture. No clearcut differences between the surfaces could be found regarding cell differentiation. By coating stainless steel samples with titanium it was evaluated in how far a difference in cell behaviour could be expected in case the samples were made of pure titanium. Strong indications were found that this is the case. In contrast to uncoated steel samples HBMC cells predominantly attached between the hemispheres and with nearly no contact with the hemispheres.

Report on the scientific results (approx. 4 – 6 pages)

Stainless steel samples

WP 1: Definition of structure characteristics and materials

WP 1 was completed, as specified in the time schedule within year 1. The same set of micro structured surfaces (50_20, 30_20, 5_20 and non-structured (NS)) was used.

WP2: Feedstock development and moulding

In order to get a complete insight of the moulding process, simulations from mould fillings for the stainless steel feedstock without nano share were calculated based on viscosity and moulding pressure data (Fig. 1). Simulations enable to look at the temperature and pressure distribution inside the mould cavity during moulding. Calculations for micron sized hemispheric surface patterned parts were not accurately possible, because of the restricted mesh-size and the very high calculation effort. Due to these problems, the surface patterns were approximated by small pyramid-shaped features. Results of moulding simulations showed normal filling behaviour of the feedstock with evenly distributed temperatures and pressures and no evidence for stress peaks.

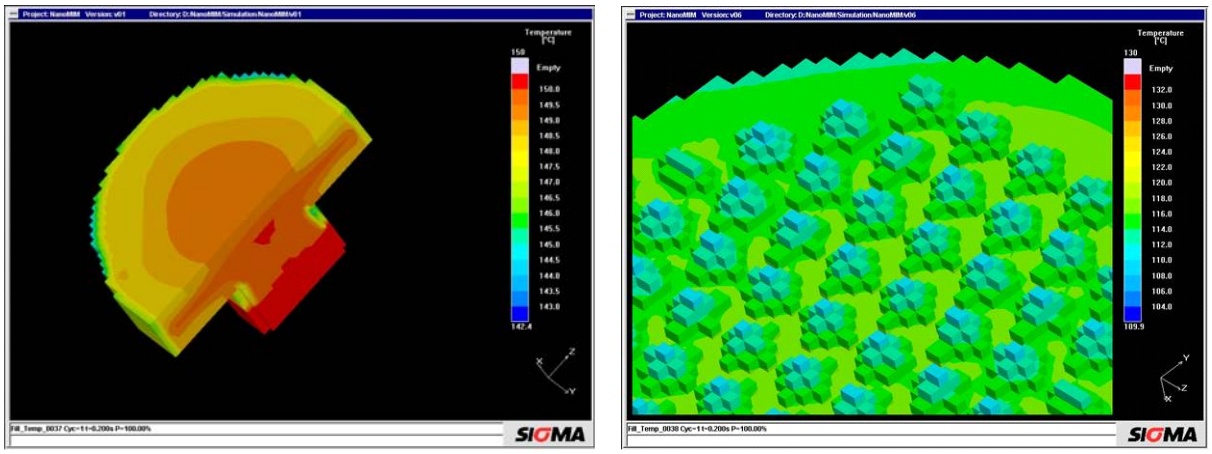

Figure 1: Calculated simulations of temperature during filling (cross section of coin shaped sample with runner and detail of microstructured surface).

WP3: Debinding and Sintering

Debinding and sintering were performed under hydrogen atmosphere with a maximum sintering temperature of 1200°C, according to the process description given in the last report.

WP4: Structure characterisation

For testing mechanical properties of the stainless steel, tensile test specimens were moulded on the injection moulding machine Battenfeld Microsystem 50. For this, feedstock without as well as with the inclusion of different shares of nano particles was used. In favour of comparability, equivalent debinding and sintering parameters as for the micro patterned coin shaped samples were used. Results of the tensile tests (Fig. 2) confirm mechanical properties of the sintered parts in the range of conventionally produced stainless steel (Yield strength > 200 MPa, Tensile strength > 500 MPa). Nano particle additions in the feedstock, lead to finer grain microstructures and consequently to a significantly increased yield and ultimate tensile strength of up to 530 MPa and 730 MPa, respectively. However, maximum elongation was reduced with higher nano powder content so that the 40 % limit, which is a typical value for 316L, was only exceeded by samples that contained no nano particles.

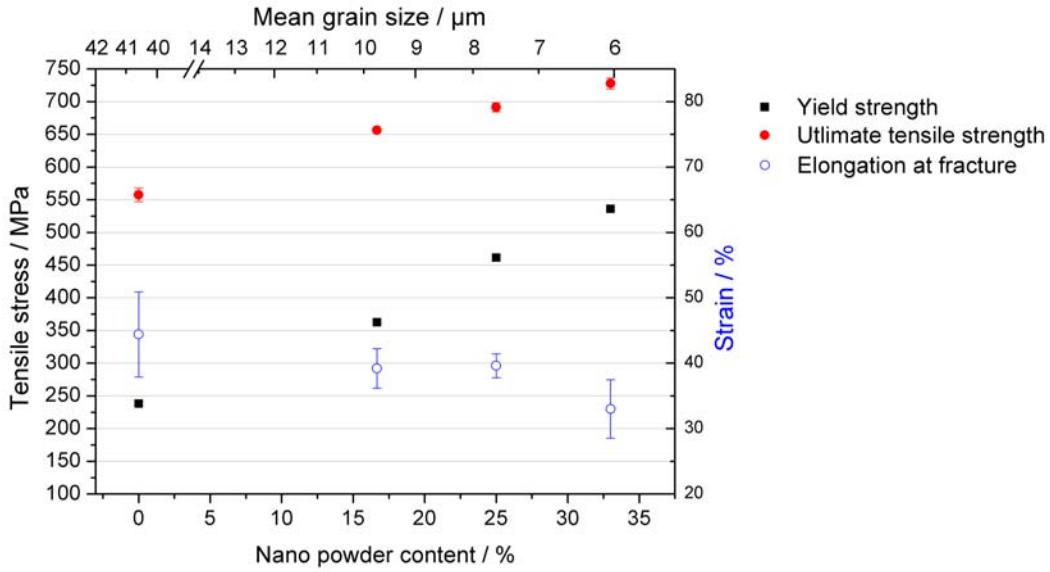

Figure 2: Results of tensile tests for stainless steel samples.

Additionally to tensile tests, also hardness measurements were conducted. Values for Vickers Hardness (HV1) of the samples were in the range of 180HV1 to 250HV1, whereas normally, values between 85HV1 and 150HV1 are common for sintered stainless steels.

WP5: Bioactivity

The evaluation of biocompatibility of materials and their surfaces is a multistep process (Fig. 3). As first step cytocompatibility has to be proven, i.e. that no toxic components are released by the material. This is generally done according the ISO10993-5 guideline by proving the non-toxicity of material extracts. The subsequent steps give an impression of the expected *in vivo* bioactivity of the material taking relevant cell cultures as tool. This is not a general issue but strongly dependent on the intended function of the implant. In case that the implant should integrate into the tissue cell adhesion, subsequent coverage of the implant surface and correct differentiation of these cells are premises. The latter is evaluated by proving good cell adherence, cell proliferation, cell differentiation and promotion of cell migration for rapid implant colonisation.

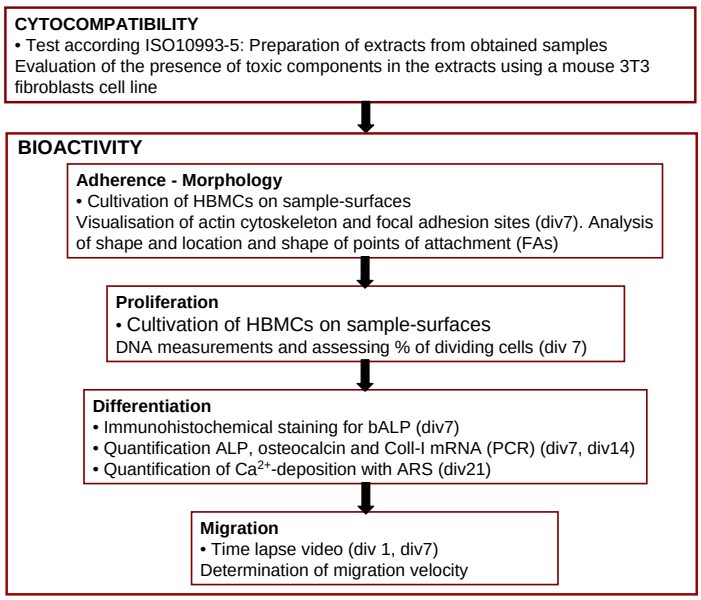

Fig. 3. Evaluation scheme of in vitro biocompatibility (cytocompatibility and bioactivity) of NanoMIM samples

The aim of the present project is to develop implants that should be osteointegrated thus in this case a differentiation of the cells towards osteoblasts is the premise. Differentiation is measured by determining mRNA and in case of ALP also the protein which presence are typical for osteoblasts differentiation, i.e. (bone specific) alkaline phosphatise (ALP) and collagen-I as early differentiation stage markers, and osteocalcin as late marker. In addition bone matrix formation is also measured taking calcium deposition as index.

Cytocompatiblity

Extracts of the obtained 316L-samples were obtained by incubation the samples for 72 hours in pH 7.4 buffered bidest water. After adding cell culture medium components 3T3 fibroblasts were treated with the extracts at different dilutions for 5 days. Therafter functionality of the cells was evaluated by quantification of total culture DNA (index of proliferation) and cellular uptake of neutral red (index of viability). No indications could be found for the presence of a toxic or bioactive component in the extracts suggesting that no such component was released by the examined samples (Fig. 3).

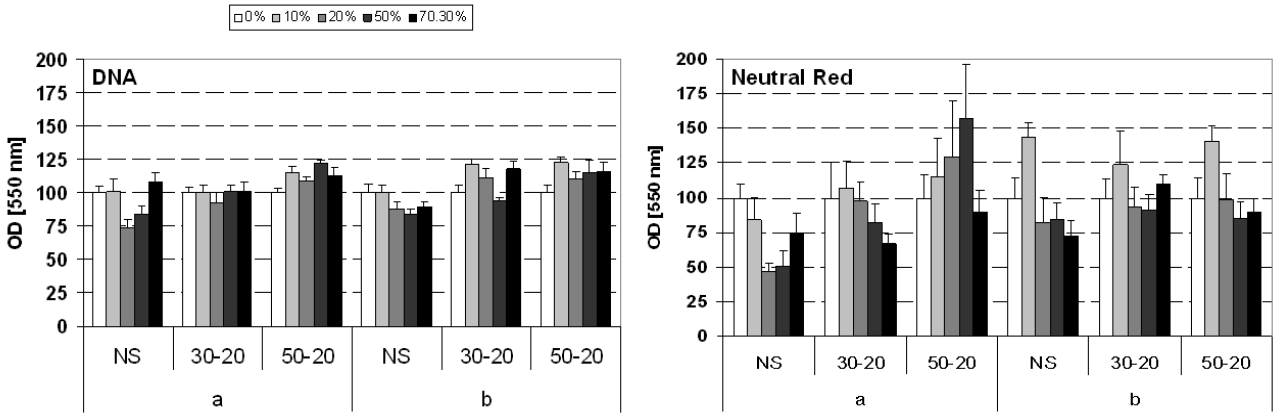

Figure 4: Biocompatibility tests taking total culture DNA and lysosomal Neutral Red accumulation as indices, showing that extracts of obtained materials prepared using both feedstock (a) without nanoparticles and (b) with 33% nanoparticles were not toxic

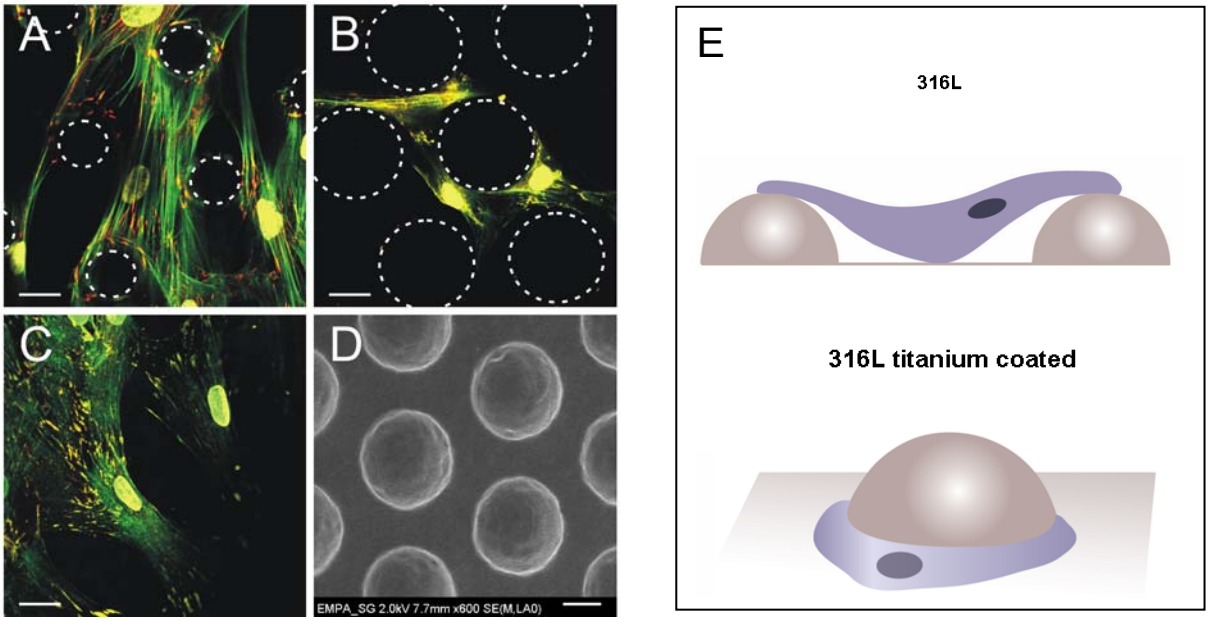

Figure 5: Confocal Laser scanning images (A-C): HBMCs cultured for 7 days onto 50_20 surfaces. Actin green; focal adhesion plaques red; nuclei yellow; scale bar 20 µm (A) Stainless steel: Cells were attached to the hemispheres with contact to the neighbouring planar surface between the hemispheres and contact with the hemisphere top. (B) Titanium coated steel: Cells were observed on the plane surface between the hemispheres. (A-B) equal magnification, but different level. (C) Planar (NS) titanium coated steel surface: Cells were well-spreaded.(D) SEM picture of the 50_20 surface. Schematic presentation of the differences in cell adhesion on structured stainless steel and titanium coated steel surfaces.

Bioactivity

Using primary human bone marrow stromal cells (HBMCs) it could be shown that cells are significantly influenced by micro structured surfaces (30_20 and 50_20 micro patterns) compared to planar controls which have the same secondary submicrometer sized structure all being prepared using the Nano-MIM process.

a. *Cell adhesion and spreading.* Cell adhesion and spreading were analysed after staining of the actin cytoskeleton using phalloidin and of the focal adhesions (FA) by staining for vinculin. On 30_20 as well as on 50_20 surfaces it was observed using CLSM and Imaris 3-D modelling software that the cells adhere predominantly to 1-3 protruding hemispheres with nearly no contact to the planar region between the protruding structures (Fig. 5A). By day 7 in culture, a significant increase in focal adhesion size was associated with the microstructured surfaces compared with the NS control. The morphological conformation of the seeded cells, as revealed by fluorescence cytoskeleton labelling, also appeared to be guided in the vertical dimension between the hemisphere bodies. Quantitative evaluation of this guidance took place using live cytoplasm fluorescence labeling and image morphometry utilizing both, compactness and elongation shape descriptors. Significant increase in cell compactness took place as a function of the hemisphere arrays indicating, possibly, a collective increase in focused cell attachment to the hemisphere bodies across the entire cell population. Micrometer scale hemisphere array patterns have therefore exhibited considerable bioactivity at least at a microscopic level (Fig. B C). No clearcut differences were seen if surface were compared made of feedstock with 33% or without nanoparticles.

b. *Proliferation.* The effects on proliferation were assessed by measuring the total culture cell number after culting HBMC's for 2 and 7 days on top of the different surfaces (Fig. 6). A significant reduction due to the presence of the hemispheres could be found. No significant differences could be found between the several samples.

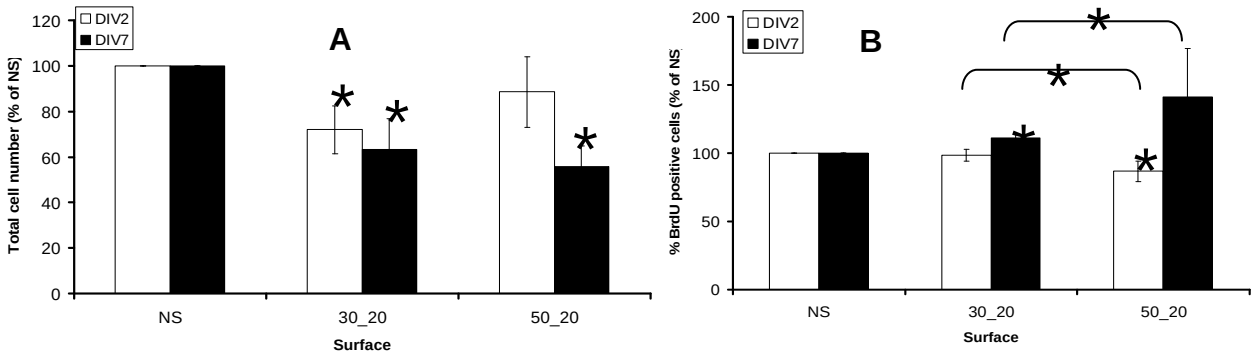

*Fig. 6. The influence of the presence of hemisheres on the total HBMC cell number (A) and percentage of cells that are dividing during the previous 24 hours (B) (BrdU positive cells) after 2 and 7 days in culture. Cell were kept in osteogenic medium. Materials were prepared using feedstock I. *:p>0.05 different from NS or as indicated in the figure.*

c. Differentiation. In a first step the effect of type of feedstock and presence of hemisphere-like structures on osteogenic differentiation was evaluated by staining cells for the presence of bone specific ALP after 7 days in culture. Here no differences could be detected. In a second step total culture alkaline phosphatise (ALP), collagen-I and osteocalcin mRNA was measured semi-qualitatively by PCR of HBMC cultured for 7 and 14 days on the various substrates. The results of ALP and collagen-I are presented in figure 7. No clearcut effect of feedstock or surface structure on ALP and collagen-I expression was found. In a third step calcium deposition as index for bone matrix formation was measured after 21 days in culture (Fig. 8). Here only small differences between the samples could be detected. However, relative to tissue culture plastic an increase could be found.

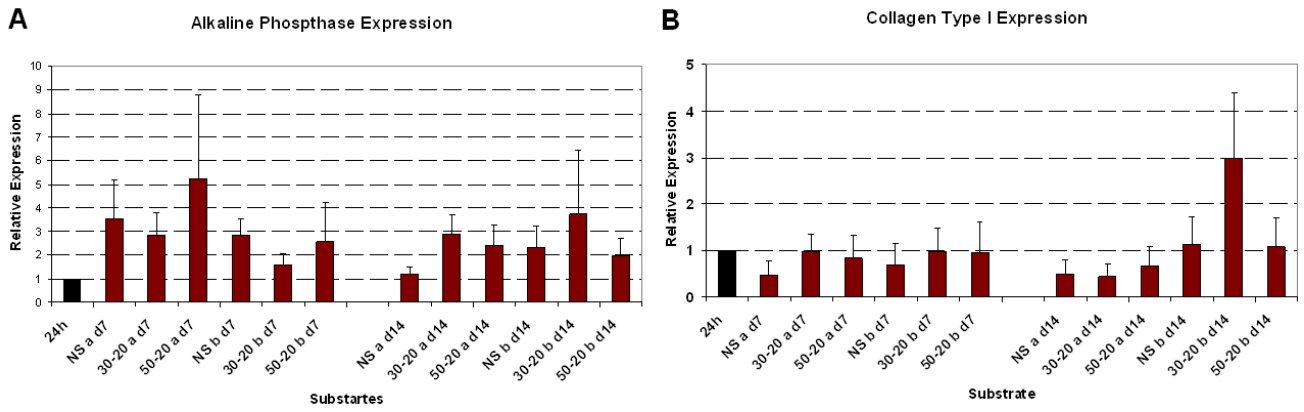

Figure 7: The differentiation of HBMCs were analysed regarding Alkaline Phosphatase (A) – and Collagen Type I (B) –expression after 7 and 14 days of cultivation on non-structured (NS) and structured (50_20, 50_20) surfaces of both feedstocks (a)(feedstock without nanoparticles) and (b) (feedstock with 33% nanoparticles). No clearcut difference in expression between the samples could be detected.

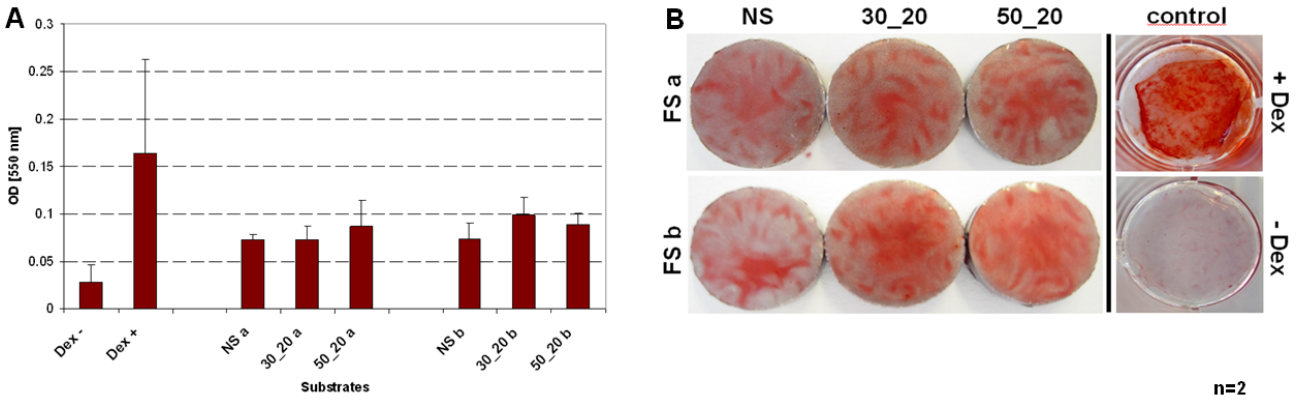

Figure 8: Bone matrix formation in HBMCs cell cultures after 21 days on non-structured (NS), 30-20 and 50-20 surfaces in osteogenic medium without dexamethasone (Dex). Calcium deposition was measured using Alizarin Red S staining as an index. Increased Ca2+-deposition was seen on non-structured (NS) and structured steel samples relative to tissue culture treated polysterol culture dish as visualised by photographic documentation as well as by photometric measurement. The deposition on 30_20 and 50_20 was only slightly enhanced relative to NS. The modification of surface roughness (feedstock without versus with nanoparticles) was without effect.

d. Migration. Time lapse videos of DiI labelled cells were made after 24 hours and 7 days taking every 15 minutes one picture. The differences in location between 2 time points of the cell centre were taken as migrated distance and base of migration velocity analysis. Frequency analysis of the measured velocities revealed no differences in migration velocity frequency patterns if comparison was made after 24 hours. However the frequency pattern of cell cultured on the micrometer structured surfaces was completely different if measured after 7 days. The velocity after 7 days was markedly increased. In contrast, the frequency pattern of cells on NS surface was similar to that of 24 hours.

WP6: Transfer of results

The results gained so far have already been presented to a broad public on several occasions, including international trade fairs, conferences and scientific publications.

Titanium samples

WP 1: Definition of structure characteristics and materials

Additional to the micro structured surfaces (50_20, 30_20, 5_20 and non-structured) a new mould with hemispheres of 100 µm in diameter was used, thus responding to the rather coarse available titanium powder particles.

WP2: Feedstock development and moulding

During the last year of the project, experiments with titanium powders were carried on.
Three different binders were tested. Starting from a binder with 25 % polymer content (binder A), as was also used for stainless steel, two variations were made to reduce oxygen and carbon contamination of the sintered titanium samples. Because of the previously good results concerning diminished contamination with carbon by reducing the polymer content of the binder to 25 %, a new binder with even further reduced polymer content of 15 % was tested, in the following referred to as "binder B". In a second step, the amount of stearic acid of this binder was reduced from 5 % to 2 % (binder C), because the carbonyl group of the acid could represent an oxygen source.
Using mixtures of the micron sized and the nano meter sized titanium Grade-1 powder, feedstocks were produced and moulded on the laboratory injection moulding machine. The feedstocks showed good mouldability, also good replication of the surface micro patterns (Fig. 9).

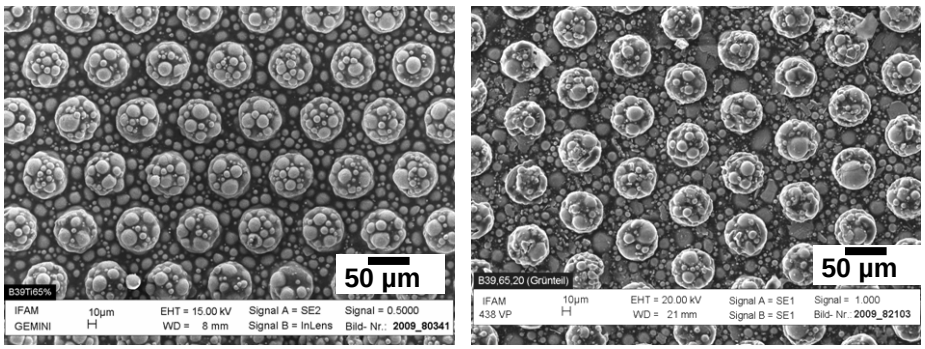

Figure 9: SEM of green parts (binder B) moulded on HEK. Left, without nano share, right: with 20 % nano share.

After the promising moulding results on the HEK, moulding on Battenfeld Microsystem 50 followed. For moulding of patterned surfaces, mould inserts with 50 µm structures and additional bigger micro patterns of 100 µm in diameter were used, due to the in comparison with stainless steel rather coarse powders. In Fig. 10 green parts with 100 µm patterns are shown prepared from feedstocks without and with 20% nano powder content. Slightly more accurate mould replication was obtained with nano powder additions.

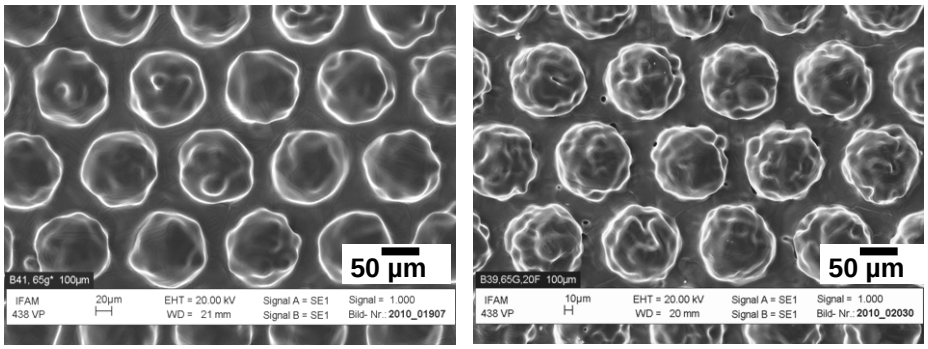

Figure 10: SEM image of green parts with replicated 100 µm pattern moulded on Battenfeld Microsystem 50. Left: feedstock with binder C without nano powder; right: binder B with 20% nano powder additions.

However, moulding of patterned surfaces with binder B turned out to be very difficult as the micro patterns were ripped off the surface during demoulding, so that a good replication of the mould was only possible on small patches of the total surface area. Going back to 25 % polymer, the amount of stearic acid was decreased, because pretests indicated that the acid could be a source for oxygen. With this newly tuned binder composition (binder C), more feedstocks were prepared and moulded. The feedstocks with 25 % polymer content were overall very good to mould and the surface patterns could be very well demoulded without getting damage.

WP3: Debinding and Sintering

Debinding and sintering were performed under argon atmosphere with a maximum sintering temperature of 1300°C, according to the process description given in the last report.

WP4: Structure characterisation

Chemical analyses of the sintered parts with 15 % polymer content and without nano particle share confirmed very low contaminations, so that overall Grade-1 samples were achieved. The achievements concerning the reduced oxygen contamination are surely also a result of the installed kneading system inside a glovebox with inert argon atmosphere. Samples made from feedstocks with a binder containing 25 % polymer had slightly increased oxygen content (Grade-3) but nitrogen and carbon contamination also correspond to Grade-1. An explanation for the higher oxygen level in these samples could be the higher amount of stearic acid inside the feedstock. Therefore investigations concerning a binder variation with 25 % polymer content and reduced stearic acid share of only 2 % instead of 5 % were carried out (Binder C). Unfortunately only marginal improvements were achieved. Hence, Binder C presents the compromise between good mouldability and as low contamination as possible. Due to the originally already contaminated nano powder and also because of the high surface area of this powder, samples with nano powder share exhibited very high oxygen and carbon contents (> Grade-4).

As can be seen on micrographs in Figure 5, it was possible to refine the microstructure significantly by additions of finer powders in the feedstock, which is similar as was found for stainless steel. However, slightly increased porosity as well as the presence of bigger pores is noticeable for the sample with nano powder additions (Fig. 11, right).

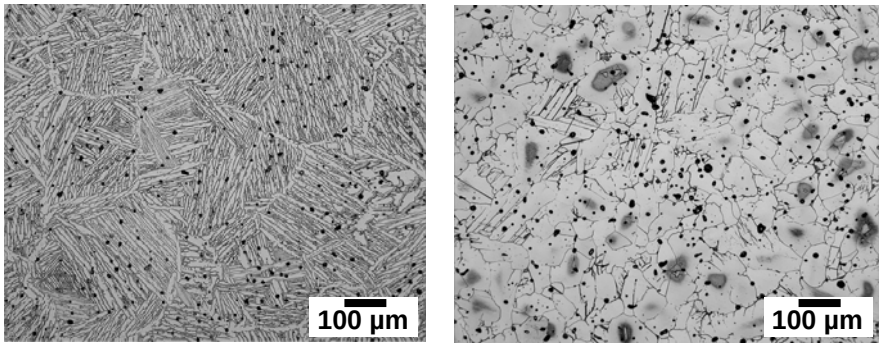

Figure 11: Micrographs of sintered non-patterned parts. Left: without nano powder; right: with 20 % nano powder. With nano powders, a refined microstructure is obtained.

White light profilometer measurements on sintered non-patterned samples revealed higher surface roughnesses than the values found for stainless steel. The roughness values for the various titanium samples were in the range of 1.06 µm to 1.29 µm. No relation between powders (grain size) or binders was obvious but a small improvement was observed when using the Battenfeld Microsystem 50 instead of the laboratory piston machine. As a preliminary mechanical test, hardness measurements were performed using Vickers. Mean values in between 185HV1 to 297HV1 were found, whereas higher values were obtained for samples with nano powder shares, and is possibly an effect of solid solution hardening due to the originally contaminated nano powder.

WP5: Cytocompatiblity and Bioactivity

Optimal samples prepared from pure titanium were not available for biological evaluation until the end of the project. To obtain at least an idea regarding in how far cells will react differently to titanium samples in comparison to steel samples, steel samples were coated with a thin layer (about 20 nm) of pure titanium using CVD deposition. XPS analysis revealed that the samples were completely coated by titanium. Titanium coated steel samples were found to be cytocompatible. Regarding cell adhesion, in comparison to the cultured cells on micro structured stainless steel (316L) surfaces HBMCs seeded on titanium coated steel samples preferred to be located in the area between the protruding hemispheres (Fig. 5 B und E).

WP6: Transfer of results

As for stainless steel, the results have been presented to a broad public on several occasions, including international trade fairs and conferences.

Self-evaluation in comparison with the original objectives and working plan (e.g. unexpected results, other deviating developments in terms of contents/methodology)

Goals mentioned in proposal vs. results

	Objections	Achievements
WP1	Stainless steel and titanium Micro-structured tool inserts Characteristics defined 3 different structures selection of best structure based on bioactive tests	completed completed completed completed completed
WP2	Glovebox Viscosity and pVT-data Simulation Sigmasoft Optical control BF Process parameters and part quality relation	completed completed completed not applied completed
WP3	TGA, DSC Higher heating rates (> 20K/min) Reduced sintering T (- 200°C to - 400°C) Microwave sintering	Sinterdilatometry instead completed completed completed
WP4	Optical laser profilometry and SEM Grain size analyses, density, pore size EDX Metal ions analyses of culture medium	White-light profilometer and SEM completed completed not needed since no toxicity was found
WP5	Cytocompatibility Cell adhesion Proliferation Migration of cells Differentiation of cells	completed completed completed completed completed
WP6	Transfer of results Publications, conferences, fairs, newsletters	completed completed

Expected main results and achievements:
The expected main results and achievements of this project were:

1. Introduction of micro- and nanostructuring into MIM as a series production process
2. Development of a new cost-effective manufacturing process for potential medical applications with bio-active surfaces
3. Enhanced material properties due to surface design that may be transferred to other application areas
4. New knowledge about the relationships between structure, bioactivity and biocompatibility of nano- and microstructured materials

With stainless steel as material, all milestones as specified in the project proposal were fulfilled in time. Overall, no critical deviations from the work plan occurred during the project. In Fig. 6, the time schedule as presented in the project proposal is shown. Overall, the project was running exactly within schedule during most of the duration. This applies especially for the investigations on NanoMIM of stainless steel. In agreement with the proposal, investigations related to titanium had started later during the project, and we needed to prolong the project duration for three months as compared to the original work plan in the third year. Some final investigations were carried out a few weeks after the official end of the project, in September 2010.

Project duration / Work package	Year 1				Year 2				Year 3			
	Q 1	Q 2	Q 3	Q 4	Q1	Q 2	Q 3	Q 4	Q1	Q 2	Q 3	Q 4
WP 1: Definition of structure characteristics and material												
WP 2: Feedstock development and moulding												
WP 3: Debinding and sintering												
WP 4: Structure characterisation												
WP 5: Bioactivity												
WP 6: Transfer of the results												

Figure 3: Time schedule as presented in the project proposal.

Added value gained through interdisciplinary and international cooperation

In the context of several opportunities IFAM and Empa continued the fruitful collaboration and further exchanged knowledge about each others fields. The following specific actions were taken:

- regular project meetings every 4-6 months
- continuous sample exchange
- concerted agreements on next steps.

These events have proven directly relevant to the constant progress in the project, as personal communication and meetings in this interdisciplinary field cannot substitute the e-mail and telephone communication, even if carried out on a weekly basis.

Research efforts in the field of biomaterials technology have been getting more significant in the last few years. The interdisciplinary collaboration between materials engineers and biologists in this project has helped to bring together two different scientific communities and to multiplying knowledge for both partners.

Future perspectives and sustainability of the project (e.g. follow-up projects, appointments)

The successful development of hierarchical micro / nano-patterning on 2-dimensional surfaces consequently raises the goals of transferring the technology to 3-dimensional parts

Besides of process development and surface characterisation, the main focus is to drive the technology towards further advanced titanium processing and to verify the effect of the new functionalised surfaces on cell behaviour in more extensive biological studies. An appropriate model is a cylindrical implant with micrometer sized hemispheres and nano-sized grain structure. *In vitro* and *in vivo* studies will give a more complete understanding of bone formation induced by surface patterning and information about the surface topographical cues which have to be included to get improved osteointegration. Financial support possibilities for this research are currently discussed. Currently cell culture experiments are going on but on the level of SE-thesis.

Contribution to the specific aims of the funding initiative

The project was funded within the initiative “innovative methods for manufacturing of functional surfaces”. Our work contributed significantly to this area as we developed the processing technology for micro patterned surfaces by metal injection moulding of nano powders. This lead to previously unique micro feadtures (protruding hemishpheres) that cannot be manufactured by downstream processing methods. Furthermore the small grain size provided tailored submicron roughness on the surfaces and enhanced mechanical performance of the bulk material. The functionality of the surfaces that was targeted, namely enhanced bioactivity, could be clearly demonstrated and validated by comparing with non-structured materials.

Public relation activities and resonance in the media

In consequence to the results obtained during the project, more and more publications with engineering aspects were published. Also an article with emphasis on the NanoMIM approach has been published in the Fraunhofer magazine “Weiter.vorn” issue Nr. 2 / 2009. This magazine highlights selected innovative projects of the Fraunhofer society. Above this, NanoMIM samples have

been presented at Hannover Fair 2008 and 2009 as well as on Medtec 2009 and 2010 in Stuttgart and on Compamed 2009 in Düsseldorf (November 18-20, 2009). Further an article “The processing of biomaterials by for implant applications by powder injection moulding” in: Powder Injection moulding International 4 (2010) 2; 49-52 was released presenting, among others, the main achievements of this project to the powder injection moulding community.

Further aspects (e.g. particularly beneficial or obstructive circumstances, experiences with cooperation)

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Part II: Tables	
Participating researchers and students, separated by institution and funding source	Scientists (IFAM): Dr.-Ing. Philipp Imgrund (FhG) Dipl.-Ing. Vera Friederici (VW-Stiftung) Scientists (EMPA): Dr. Arie Bruinink (EMPA) Dr. Malak Bitar (VW-Stiftung) Claudia Brose (VW-Stiftung) Dr. Elena Burguera (Spanish government, VW-Foundation) Student (IFAM): Selina Seefried (VW-Stiftung) Student (EMPA): Fausta Benini (VW-Stiftung)
Additional cooperation partners in the project (not applicants)	
Theses written in the course of the project (Diploma, MA, MSc, PhD, Habilitation, including name of researcher responsible)	1. V. Friederici: Untersuchungen zum Mikro-Metallpulverspritzgießen von biokompatiblen Werkstoffen mit ultrafeinen Pulvern, Diploma (conducted at IFAM), Technical University Darmstadt, 2008 2. S. Seefried: Funktionalisierung von Titan-Oberflächen durch Mikro-Metallpulverspritzgießen zur Verbesserung der Zelladhäsion, Diploma (conducted at IFAM), Hochschule Bremerhaven, 2009 3. F. Benini: A novel method for surface functionalisation of orthopaedic implants: Human bone marrow stem cell reactions, MSc, 2009
Publications (Please include reprints of publications directly related to the project.)	1. Ph. Imgrund, V. Friederici, N.Salk, A. Bruinink, M. Bitar: Surface Structuring by Micro-MIM – Technology and Applications. In: Advances in Powder Metallurgy and Particulate Materials 2008, Eds: R. Lawcock, A. Lawley, P. McGeehan, Metal Powder Industries Federation (2008) pp. 4-230 – 4-235. 2. Burguera EF, Bitar M, Bruinink A (2010) Novel in vitro methodology to investigate heterotypic cell-cell interactions. *Eur. Cells Mat*. 19, 166-179 3. Friederici V, Bitar M, Imgrund Ph Bruinink A, (2010) Micro metal injection moulded stainless steel materials with defined micrometer and submicrometer surface features for medical applications. Process development and material characterization *Eur. Cell Mat. (submitted)* 4. Bitar M, Friederici V, Imgrund P, Brose C, Bruinink A (2010) Micro metal injection moulded stainless steel materials with defined micrometer and submicrometer surface features for medical applications.

	Biocompatibility and effects on human bone marrow stromal cell attachment and 3D morphology. *Eur. Cell Mat. (submitted)* 5. M Bitar, F. Benini, V. Friederici, P. Imgrund, A. Bruinink: Human Bone Marrow Stem Cell Motility and Proliferation as a Function Hemisphere-Array Metal Surface Patterning (in preparation)
Specific events, e.g. workshops (Program, list of participants, abstracts and other details may be appended in a separate file.)	1. Hannover Messe Industrie 2008; April 21-25, 2008, Hall 16, booth F16 (exposition of micro-structured parts) 2. 1st International Symposium on Functional Surfaces, Bremen, Germany. 18.-19.06.2008. 3. Hannover Messe Industrie 2009; April 20-24, 2009, Hall 6, booth E16/B3 (exposition of NanoMIM parts) 4. MedTec, 2009, March 3-5, Hall 5, booth 1522, Stuttgart, Germany (exposition of NanoMIM parts) 5. Compamed 2009, November 18-20, 2009, Düsseldorf, Germany (exposition of NanoMIM parts) 6. MedTec, 2010, March 23-25, Hall 6, booth 1522, Stuttgart, Germany (exposition of NanoMIM parts)
Abstracts directly related to the project (title)	1. M. Bitar, P. Imgrund, A. Rota, F. Petzoldt, A. Bruinink: Controlled Surface Functionalisation of Orthopaedic Implants: In-vitro Osteogenic Bioactivity 14th Swiss conference on Biomaterials, Basel, Switzerland. 08.05.2008. (poster). 2. M. Bitar, P. Imgrund, A. Rota, A. Bruinink: Combined Micro-Nano Patterning for Endosseous Implants: Investigating Osteoblastic Cell Responses In vitro; 8th World Biomaterials Congress, Amsterdam, the Netherlands. 28/05-1.06.2008 (oral presentation). 6. Ph. Imgrund, V. Friederici, A. Bruinink, M. Bitar: Manufacturing of biomedical components by powder-based processing technologies; 03.09.2008; MSE Conference, Nürnberg, Germany (oral presentation) 7. Ph. Imgrund, F. Petzoldt, V. Friederici: Micro-MIM for medical applications. Proc. of EuroPM 2008 Congress, Mannheim, Germany. EPMA, Shrewsbury, Vol. 2, pp. 305-310 (oral presentation) 8. V. Friederici: Strukturierung von Implantatoberflächen durch Mikro-Metallpulverspritzgießen; 19.11.2008, Regionaltagung des Regionalverbundes Mikrosysteme für die Biotechnologie - Nord (MBN), des Arbeitskreis BioMST und Lifesciences e.V., Bremen, Germany (oral presentation) 9. M. Bitar, V. Friederici, A. Rota, P. Imgrund, A.

	Bruinink: Controlled Topography Functionalisation of Metal Surfaces: The Impact on Marrow Stromal Cell Focal Adhesion and Morphological Conformation; European Society of Biomaterials, Lausanne, Switzerland, 2009 (oral presentation) 10. V. Friederici, P. Imgrund, M. Bitar, A. Bruinink: Micro/sub-micro –surface structuring of implant material through metal injection moulding of hybrid micro / nano powder mixtures ; European Society of Biomaterials, Lausanne, Switzerland, 2009 (oral presentation) 11. V. Friederici, P. Imgrund, M. Bitar, A. Bruinink: Tailoring of implant surfaces for enhanced cell performance by µ-MIM; EuroPM, Copenhagen, Denmark, 2009 (oral presentation 12. V. Friederici, Ph. Imgrund, A. Bruinink, C. Brose, M. Bitar: Metal injection moulding for producing stainless steel and titanium implant surfaces with defined micrometer and submicrometer structures for enhanced cell performance; Material Science and Engineering, Darmstadt, Germany, 2010 (oral presentation)
Patents directly related to the project (including name, patent number)	"Biokompatibles Bauteil und Verfahren zu dessen Herstellung – DE 10 2008 008 219.8", filed for PCT, February 2009 by Fraunhofer Gesellschaft zur Förderung der Angewandten Forschung e.V.