Final publishable summary report

(Text only – without figures)

Executive summary

The aim of Bio-Scaffolds project was the development of novel breakthrough technologies for the 3D fabrication of individualized implants in the field of orthopedics, through the application of novel bio-inorganic materials which are regeneratively active. Such materials/implants are urgently needed in our aging societies.

Intensive studies have been performed to develop a new generation of implants that allow the regeneration of functional bone tissue. The materials/scaffolds for such implants must be not only osteoconductive (attracting and guiding cells to the lesion) but also osteoinductive (inducing bone precursor cells to become active osteoblasts). Ideally they should have the capacity to induce the formation of new bone, by the body's own cells, and, by that, directly contribute to bone remodeling.

By learning from nature, and following the rational that drives evolution, and the fact that all metazoans evolved from a common ancestor, the sponges, members of our consortium introduced novel bio-inorganic materials with the potential to be suitable for bone tissue engineering. These materials comprise: *(i)* Bio-silica, a material that forms the inorganic skeleton of the siliceous sponges and turned out to be morphogenetically active, cell embedding, self repairing and self adapting in human cells. *(ii)* Biogenic polyphosphate (bio-polyP), an inorganic polymer that is also morphogenetically active and consists of long chains of phosphate units linked by high-energy bonds. This project revealed that both inorganic biopolymers can be applied for the fabrication of customized scaffolds/implants for bone tissue engineering/repair, either alone or in combination that are biocaompatible, biodegradable and regeneratively active.

The main results of Bio-Scaffolds project are: *(i)* The methodology for medical imaging, interfacing, and CAD/CAM processes for the fabrication of customized scaffolds by rapid prototyping has been developed. HIGHLIGHTS were the development of a software to import CT and MRI images that can be visualized in full 3D, a navigation system for correct implant placement, and a system for planning, guiding and monitoring surgical intervention. *(ii)* As a novel component of bioactive bone regeneration scaffold materials, the two morphogenetically active bio-inorganic polymers, bio-silica and bio-polyP, have been introduced. HIGHLIGHTS included the functional dissection of the biosilica-forming enzyme, silicatein, the successful application of the recombinant silicatein in 3D cell printing, the successful development of a method for hardening of bioprinted scaffolds, and a novel morphogenetically active Ca-polyP material for bioprinting applications. *(iii)* A smart micro-unit system consisting of microgranules from nanopowder with microchannels for nutrient delivery has been developed. HIGHLIGHTS were the successful synthesis of hydroxyapatite (HA) nanoceramics with particle size below 30 nm and the preparation of the micro-units from HA via dip-coating of polymeric fibers and sintering. *(iv)* Two material solutions for the fabrication of customized scaffolds have been established: additive manufacturing (robocasting, 3D printing, surface-selective laser sintering) and subtractive fabrication (3D milling). HIGHLIGHTS were the development of novel biodegradable ceramic-polymer composites by deposition of natural polymers and PCL/HAp nanocomposite coatings onto robocast scaffolds, a novel bioprintable material based on polyP and modified chitosan, and the demonstration of the morphogenetic activity of the scaffolds.

(v) The scaffolds have been functionalized in order to achieve optimal combination of mechanical performance and biological properties. HIGHLIGHTS were the modification of (robocast) ceramic scaffolds and the demonstration of the enhanced biological performance of Ca-polyP or bio-silica coated robocast scaffolds. *(vi)* The unique ability of enzymatically formed bio-silica to encapsulate cells without affecting cell viability and function has been successfully exploited. HIGHLIGHTS were the successful 3D printing of cells embedded into a bio-silica or bio-polyP-containing alginate hydrogel. *(vii)* The microstructural, mechanical and biological properties of the generated scaffolds have been characterized both in vitro and in vivo. HIGHLIGHTS were the demonstration of the enhanced *in vivo* performance of HAp robocast scaffolds by biosilica coatings, the advantageous mechanical properties of the biosilica- and polyP-containing scaffold materials and cell-containing hydrogels, the increased expression of growth factors/cytokines and the enhanced HA formation by cells growing on the newly developed 3D printed scaffolds. Animal implant experiments revealed highly promising results for microspheres containing encapsulated silicatein and silica (rabbit patellar groove model for cancellous bone healing), as well as for the new polyP-containing scaffold (rat calvarial defect model). *(viii)* Patent relevant results have been identified and new patent applications filed. *(ix)* There was an extensive transfer of technical skills and know-how between the European and Chinese partners. *(x)* The new knowledge generated has been disseminated both to the scientific community and to potential endusers, as well as to the broader public. This project resulted in more than 30 publications in high-impact journals. In addition, organization of two summer schools and two workshops, and, as HIGHLIGHTS, the co-organization of three international conferences (two CEITEC Conferences “Frontiers in Life and Materials Sciences” in 2014 and 2015, Brno, Czech Republic, and 1st International Conference on “3D Printing in Medicine”, Mainz, Germany, 2016). HIGHLIGHTS of the communication activities were an exhibition in Mainz Museum and a Public Night Lecture (Mainz University Medical Center). In addition, participation in industrial fairs (Medtech Rhineland-Palatinate 2014 and 2015 and CHINA HI-TECH FAIR 2014, Shenzhen, China).

Summary description of the project context and the main objectives

The main goal of the Bio-Scaffolds project was the development of new solutions and a corresponding material production chain for the fabrication and application of customized scaffolds for the repair of bone defects.

These scaffolds should exploit the unique ability of two bio-inorganic materials, bio-silica and biogenic inorganic polyphosphate (bio-polyP) to be morphogenetically active and to induce new bone formation.

To achieve this goal, the following specific project objectives had to be accomplished:

- To extract and transform the data from medical imaging (MRI and CT) for fabrication of customized scaffolds by rapid prototyping, including the development of *(i)* a CAD/CAM planning software package; *(ii)* a protocol for patient scanning (MR and CT) that will facilitate customization of patient scaffolds; *(iii)* an interface between imaging data and CAD/CAM planning; and *(iv)* an interface between CAD/CAM planning and production step.

- To develop smart micro-unit systems for rapid prototyping, consisting of microgranules from nanopowder with a microchannel through it for a nutrition delivery, as well as scaffold materials based on specific enzymes, silicateins,

which are able to form bio-silica enzymatically, and in particular, to develop novel composite materials consisting of the morphogenetically-active polymers bio-silica or bio-polyP or combinations.

- To realize strong and rigid 3D scaffolds applying the rapid prototyping process, mimicking the natural nanoscale architecture of the extracellular matrix (ECM), by implementing the micro-units or the bio-silica or bio-polyP containing materials in a rapid prototyping process or by applying bio-silica coatings, either alone or in combination with bio-polyP, onto bioceramic and bioactive glass scaffolds fabricated by SFF techniques.

- To develop and realize two complementary procedures in addition to the rapid prototyping techniques: *(i)* 3D printing of cells embedded in a morphogenetically active and controlled biodegradable biosilica-containing alginate hydrogel matrix, and *(ii)* 3D milling of porous gelcast foams and SFF performs.

- To characterize the physical and physiological properties of the novel scaffold materials, including the characterization of the microstructural, material and mechanical properties, as well as the morphogenetic activity of these scaffolds both in vitro (cell culture experiments) and in vivo (animals experiments; partially performed by the Chinese partners).

- To establish a proper management of intellectual property and to promote an efficient transfer of technology within the consortium, including the identification of products of potential industrial interest and filing of patents**.**

- To disseminate and communicate the knowledge generated within the project and to train the team members of the project partners in the technical skills / know-how available in the consortium, including publications in refereed scientific journals, **p**resentations at scientific meetings, workshop organization, preparations of leaflets, brochures and CD-Roms, contributions to media (press, broadcast etc) and project website.

- To establish and efficient project management structure, including close links between the European partners and the Chinese partners involved in this project.

Description of the main S&T results/foregrounds

The main S & T results of the project can be summarized as follows:

1. IMAGING AND CAD/CAM PLANNING

Summary:

The data extraction from medical imaging (MRI and CT) and transformation was performed by the Chinese partner **THU-2**. **ORTOMA** has developed the IT system for planning, implementing, and monitoring of the entire surgical process, including *(i)* a communication interface with a commercial hospital PACS server system; *(ii)* a voxel-based OpenGL CAD software platform for true 3D volume data visualization; *(iii)* the separation and repositioning of bones function (Range of Motion simulation); and *(iv)* a module for generating CAD templates component. In addition, **ORTOMA** developed a data export functionality to deliver software packages for rapid prototyping.

These results brought considerable progress compared to the state-of-the-art because *(i)* existing software for planning and placing implants on the market are mainly based with 2D functionality; *(ii)* no software is on the market that has the ability to enable monitoring of the complete treatment process; *(iii)* no planning software is on the market that is built with a general function with support for any implant manufacturer; and *(iv)* no software is on the market that has the capability to combine volume rendering visualization, 3D planning, CAD functionality and file formats for production purposes (CAM).

In detail:

Extracting high resolution 3D image data from CT and MRI: This work has been done by the Chinese consortium (partner **THU-2**); see below (Summary of the results from Chinese consortium).

CAD/CAM planning software package: ORTOMA developed a module for generating CAD templates components. Individualized scaffold 3D components with CAD-generated microstructures can be imported and positioned. Data preparation and export functionality to external CAM systems (Additive Manufacturing) has been developed (e.g. for use with 3D printers). In the **ORTOMA** CAD software, the preoperative clinical situation can be visualized. These functions are implemented in full 3D: measurements, clip-planes, for visual purposes during the planning stage. General tools are available to analyze the patient's anatomy, for medical decisions in advance of surgery. A data base structure has been prepared to make it possible to build up catalogue of surgical tools, scaffold structures and desired help objects. In addition, **ORTOMA** provided a protocol for patient scanning (MR and CT) that facilitates customization of patient scaffolds.

The **ORTOMA** Hip Plan software and Hip Guide has obtained a CE-certificate by a Notified Body. The CE-certificate is required in order to release the Ortoma Hip Plan 3D software and Ortoma Hip Guide to market and clinicians, in order to initiate clinical studies and market approach. The **ORTOMA** Hip Plan is an orthopedic software application for preoperative planning prior to surgery. The **ORTOMA** Plan includes a powerful software package containing front-end 3D visualization technologies. An illustrative 3D image of the unique patient´s anatomy is created after the surgeon has

uploaded the Medical Imaging Data set from Computer Tomography (CT) or Magnetic Resonance (MR). It is built with integration to external systems and hardware (i.e. PACS).

During surgery, the **ORTOMA** system will keep track of the surgical tools position in real-time, in relation to the patient anatomy and the virtually planned prosthetic components to be insterted. The surgeon will receive visual information, measurements and angle information about the position of the physical component in relation to the defined virtually planned position of the CAD component. The system also supports to guide the surgeon for different types of interventions performed in the patient e.g. inscisions, cutting guides removing material from the patient. The system has the possibility to support functions built into the current platform such as segmenting, planning and CAD functionality, in relation to the patient anatomy for e.g. scaffolds. The result can be exported to a 3D format supported by various additive manufacturing methods or subtractive methods.

A student report has been presented by engineer students at the Production and Production Development Department, University of Chalmers, Gothenburg, Sweden. In total five operators (students) performed the study. The students did not have any experience from the orthopaedic field, nor navigation systems. The aim was to evaluate the accuracy of the Ortoma Treatment Solution system. In total 14 hip models obtained from patient CT-scans were segmented and exported to STL files and printed by a 3D printer, representing the “real” patient. The five operators performed the full sequence utilizing the Ortoma Hip Plan software and Ortoma Hip Guide hardware on the 14 patient models. In total 4800 measurements were performed. The mean value accuracy result was 0.12 mm. The results fulfil the expectations of the systems deviation threshold.

ORTOMA have worked in close collaboration with university clinics during the development phase. The planning software has been used in clinical studies that are currently submitted by researchers at University Hospital of Skane, Sweden.

Interface between imaging data and CAD/CAM planning: A communication interface has been implemented with a commercial hospital PACS server system. From the PACS system software it is possible to import CT and MRI examinations of patients for the clinicians in a familiar environment. In the **ORTOMA** CAD software, the preoperative clinical situation can be visualized. The surgeon can use **ORTOMA** Plan to measure and define CAD implants positions in relation to the patient anatomy.

Interface between CAD/CAM planning and production step: The result from the CAD can be exported, suitable for e.g. additive manufacturing. Additive manufacturing machines has standard programs that are able to produce production data directly from the volume data, usable for medical purposes. All necessary functions for production ready data are available in the **ORTOMA** platform. The software incorporates export functionality of common file formats used for additive manufacturing.

- **Highlights**:

  - **Software has been developed to import CT images and segmented MRI images that can be visualized in full 3D - implemented (ORTOMA)**

  - **Results from the CAD can be exported for additive manufacturing – implemented (ORTOMA)**

  - **Software for guiding and monitoring surgical intervention has been**

developed

- **Data base structure has been successfully prepared to build up catalogue of surgical tools – implemented (ORTOMA)**
- **Separation and repositioning of bones: Movement simulation between patient anatomy in connection to planned 3D objects has been implemented (ORTOMA)**
- **A matching protocol has been developed to align the physical patient to the virtual patient coordinate system by means of a measurement pen, camera system and alignment software.**
- **ISO 13485 certificate obtained for ORTOMA. CE-certificatates have been obtained for Ortoma Hip Plan and Ortoma Hip Guide.**
- **System for planning, guiding and monitoring surgical intervention has been developed.**
- **Import function for DICOM data and various CAD file formats e.g. STL files, segmenting, planning, CAD reconstruction, exporting file format for additive manufacturing has been developed.**
- **ORTOMA have worked in close collaboration with university clinics during the development phase. The planning software has been used in clinical studies that are currently submitted by researchers at University Hospital of Skane, Sweden. The Ortoma Treatment Solution has been evaluated in a surgical environment during a patient hip surgery.**

2. BIO-INORGANIC POLYMERS AND MICRO-UNITS

Summary:

As a new material for the application in the rapid prototyping chain, **BUT** successfully developed nanoceramics based on hydroxyapatite nanopowders with particle size below 30 nm. The nanopowders were formed by sol-gel precipitation technique. The prepared powder was applied during dip-coating process. Micro-units from hydroxyapatite (HA) were prepared via dip-coating of polymeric fibers and sintering. In addition, **BUT** succeeded to develop a second version of a prototype dip-coating apparatus suitable for preparation of the smart micro-unit systems with a microchannel for a nutrition delivery. This version allows coating of hundred metres of very thin fibres and novel microscopy imaging analysis unit was designed for control of the process. The micro channels inside micro units have an inner diameter below 12 or 36 μm. Furthermore, hollow fibers with extremely thin walls were prepared from various materials and unique ability of the dip-coating machine was tested. **UMC-Mainz** succeeded to develop and to provide the proof-of-concept in 3D printing of two morphogenetically active polymers: *(i)* bio-silica and *(ii)* bio-polyP, as well as of combinations of both inorganic polymers. Bio-silica is formed by the enzyme silicatein; the optimal chain of the chemically synthesized polyP is 40 phosphate units. The structure-forming and structure-guiding activity of silicatein relevant to its use in 3D printing could be dissected. The recombinant proteins were expressed in *E. coli* and purified by affinity chromatography (**NANOTEC**). Furthermore, **UMC-Mainz** presented a novel method for hardening bioprinted cell-containing alginate/gelatine scaffolds using a Ca-polyphosphate/agarose overlay. **UMC-Mainz** also prepared for the first time scaffolds which are morphogenetically active, using bio-silica (synthesized

enzymatically *via* silicatein). Moreover, **UMC-Mainz** was able to demonstrate that the hardness of bioprinted alginate/gelatin hydrogels containing SaOS-2 cells can be markedly increased by addition of calcium-polyphosphate. Various 3D silica-impregnated scaffolds produced by the layer-by-layer 3D printing technique have been fabricated by **UMC-Mainz** and **NANOTEC.** In addition, **UMC-Mainz** developed two additional novel morpho-active materials: Ca-polyphosphate nano/microparticles for bioprinting applications.

These achievements brought significant progress compared to the state-of-the-art at project start because these new developments allow *(i)* omission of - expensive and not printable (heat sensitive) - growth factors and cytokines which have to be added to conventional, inert scaffold materials after 3D printing to induce new bone formation – the morphogenetically active materials are able to induce the expression of these factors/proteins by themselves; and *(ii)* the adjustment of the appropriate hardness of the scaffold materials.

In detail:

Development of new nanoceramics: The development of new nanoceramics in the project is generally based on synthesis of nanopowders as a source of starting materials for shaping and sintering. Commercially available bioceramic powders have main limitations in the available chemical compositions. Moreover, particle size is usually larger than 100 nm and strong aggregates are present. Two main directions were followed so far: *(i)* preparation of defined hydroxyapatite nanopowders, and *(ii)* preparation of bioglass. Nano hydroxyapatite powders were synthesized at **BUT** by sol-gel precipitation synthesis from inorganic precursors (calcium nitride, di-ammonium hydrogen phosphate, ammonium hydroxide). The prepared powder has particle size below 30 nm and BET surface area around 65 m^2/g. In addition, the preparation of bioglass by sol-gel precipitation was tested.

Development of smart micro-unit systems with microchannels for a nutrition delivery: The preparation of the smart micro-units is based on tailored dip-coating of a selected fibre. The main challenge during the project was the transfer of laboratory scale preparation to pilot operation. The first version of the prototype was successfully tested. High stability and reproducibility of the process was achieved.

The second version of the prototype, developed by **BUT**, allows coating of hundred metres of very thin fibres, which is scale of pilot operation. Our dip-coasting process is based on water as a solvent with a perspective of environmentally friendly processing without any toxic component. **BUT** used commercially available HA to get stable and high volume supply of bioceramics powder for elementary testing of fibre – suspension systems.

Two kind of coated fibers were applied to template micro units – para-aramid (Twaron) with diameter 12 µm and ultra-high-molecular-weight polyethylene (Dyneema) with diameter 36 µm.

Three main parameters were changed during the dip-coating: *(i)* suspension, *(ii)* fiber and *(iii)* speed of dip-coating. As the most important parameter for forming of droplets the speed of dip-coating (i.e. the speed of pulling the fiber from the suspension) was identified. After drying, the coated fibers were sintered at 950 to 1150°C.

Development of morphogenetically-active polymers consisting of bio-silica or bio-silica/bio-polyP combinations: Two morphogenetically-active inorganic polymers for 3D printing have been developed and the proof-of-concept delivered by **UMC-Mainz** and **NANOTEC**: bio-silica and bio-polyP. Bio-silica is formed enzymatically (enzyme:

silicatein), while for the second inorganic bio-polymer, the chemically synthesized polyP (preferred chain length: 40 phosphate unity) can be used. Based on these inorganic polymers, **UMC-Mainz** also succeeded to develop a method for hardening of scaffold material.

Silicatein has two functional activities relevant to its use in 3D printing: structure-forming and structure-guiding activity. **UMC-Mainz** succeeded to dissect both functions. This partner constructed a gene (*SUBDOSILICa_Q/S*) encoding for a mature enzyme which comprises instead of the S-stretch a Q-stretch; this modification resulted in an exchange of the silica-binding property by an increased capacity to form hydrogen bonds with other silicatein (protein) molecules.

To make use of the structure-guiding function of silicatein **UMC-Mainz** mutated the cleavage site within the primary translation product at the border between the pro-peptide and the mature enzyme, Q/D by Q/Q. The resulting recombinant non-processed silicatein (SILICAaNP_SUBDO) was used as a structure-guiding platform onto which soluble enzymatically active SILICAaQ/S_SUBDO was layered onto. **UMC-Mainz** demonstrated that the enzymatically active, structure-forming silicatein, SUBDOSILICa_Q/S, coated around printed non-enzymatically acting structure-guiding silicatein SILICaNP_SUBDO, has the property to synthesize biosilica.

In addition, **UMC-Mainz** developed a polyP-based material suitable for bioprintable morphogenetically active scaffold materials. This material consist of morphogenetically active Ca-polyphosphate nanoparticles and microparticles. It can be fabricated under mild reaction conditions, at room temperature. The material shows a considerable hardness (elastic modulus) of about 1.3 GPa.

The Ca-polyP granules were found to be biologically active and induce the marker gene of osteoblast formation, the alkaline phosphatase. This material is prone to hydrolytic degradation during *in vitro* incubation of the cells, suggesting that this property is associated with the observed bioactivity.

Hardening of materials for rapid-prototyping based on bio-silica/bio-polyP: One strategy to improve the mechanical strength of scaffolds prepared from bio-silica or bio-silica/bio-polyP combinations may be the use of aluminate cement. **UMC-Mainz** succeeded to develop another, biological procedure to harden cell-containing alginate/gelatine scaffolds: This partner demonstrated that addition of an agarose overlay, containing the calcium salt of polyphosphate [polyP•Ca^{2+}-complex], onto bioprinted SaOS-2 cells (bone forming cells) embedded into an alginate/gelatine matrix causes a substantial increase in the hardness of the alginate/gelatin hydrogel.

To determine the mechanical properties of the produced scaffolds, **UMC-Mainz** determined the hardness using a newly-developed nanoindenter with a cantilever that had been fabricated on the top of a ferruled optical fiber. Based on the load-displacement curves the reduced Young's modulus was calculated.

In the absence of polyP•Ca^{2+}-complex the reduced Young's modulus of the hydrogels was found to be lower than in hydrogels containing the polymer. The reduced Young's modulus for the alginate/gelatin hydrogel is 13 – 14 kPa. This value drops to 0.5 kPa after incubation of the cell containing scaffolds for 5 d. In the presence of 100 µM polyP•Ca^{2+}-complex, the reduced Young's modulus increases to 22 kPa. The hardness of the polyP•Ca^{2+}-complex containing hydrogel remains essentially constant if no cells are present in the matrix, but it drops to 3.2 kPa after a 5 d incubation period in the presence of bone-forming SaOS-2 cells, indicating that polyP•Ca^{2+}-complex becomes metabolized/degraded by the cells.

Development of silicatein/bio-silica- or silicatein/bio-silica/polyP-coated micro-units using PEG-hardened bio-silica as a "bio-glue": UMC-Mainz, in cooperation with **NANOTEC**, fabricated 3D printed scaffolds using the ZPrinter 450 (Z Corporation). The blocks were prepared layer-by-layer from Ca-sulfate hemihydrates. The powder was solidified by ink spread onto the Ca-sulfate powder. The layers were then impregnated by a short spaying cycle with Mg-sulfate. To impregnate the grids with Ca-phosphate, the grids were incubated with $CaCl_2$ and then submerged in a Na-phosphate solution. The silica impregnation of the Ca-sulfate grids was performed with prehydrolyzed TEOS (tetra-ethoxy-silane). **UMC-Mainz** applied this and further procedures based on impregnation of nanoceramics powders by dip-coating with (Glu-tagged) silicatein and incubation with substrate (pre-hydrolyzed tetra-ethoxy-silane; TEOS).

- **Highlights**:

  - **Biocatalytically active recombinant silicatein has been used for the first time in 3 printing and 3D cell printing (bioprinting) (UMC-Mainz and NANOTEC)**

  - **For the first time, dissection of the two functional activities of the biosilica-forming enzyme silicatein: (i) structure-forming and (ii) structure-guiding activity (UMC-Mainz)**

  - **First successful application of morphogenetically active scaffolds in 3D printing of bone implants (UMC-Mainz)**

  - **Development of a novel, biological method for hardening bioprinted cell-containing alginate/gelatine scaffolds by a Ca-polyphosphate/agarose overlay (UMC-Mainz)**

  - **First-time application of a method allowing hardness measurements during new bone formation on scaffolds in solution, relevant for the *in vivo* situation (UMC-Mainz)**

  - **Novel bioprintable, biodegradable and morphogenetically active hydrogel scaffold based on polyP/CM-chitosan/alginate that can be hardened in a controlled way (UMC-Mainz)**

  - **Novel morphogenetically active nano/microparticulate material: Ca-polyP nanoparticles/microparticles (UMC-Mainz)**

  - **Synthesis of hydroxyapatite nanoceramics with particle size below 30 nm was successfully accomplished (BUT)**

  - **Special dip-coating machine was successfully designed during this project to achieve productive, reproductive, and economical production process (BUT)**

  - **Micro units from hydroxyapatite were prepared via dip-coating of polymeric fibers and sintering (BUT)**

  - **The prepared microgranules contain open microchannels, which supposed to be used for nutrition delivery, with an inner diameter below 12 or 36 µm after sintering and open porous microstructure (BUT)**

3. Rapid Prototyping

Summary:

Three different methods for fabrication of 3D scaffolds were applied by the partners: *(i)* the robocasting method by **UEX**, *(ii)* the 3D ink-jet printing method by **UMC-Mainz**, and *(iii)* the method of selective laser sintering by the Chinese partner, **THU-1**. **UEX** developed novel procedures for preparation of ceramic/polymer composite scaffolds; the bioceramic scaffolds were impregnated with biodegradable polymers to increase the strength and toughness of the material. **UEX** coated 45S5 bioglass with natural biodegradable polymers and PCL/nanoHAp coatings onto similar bioglass scaffolds to produce hybrid ceramic/polymer nanocomposites with both improved mechanical properties and enhanced *in vitro* performance. In addition, **UEX** demonstrated the feasibility of the incorporation of the microunits to the rapid prototyping process but assessed the process to be impractical for the fabrication of three dimensional scaffolds. Instead, scaffolds with continuous internal channels were developed. **UMC-Mainz** and **NANOTEC** applied the method of 3D printing for preparation of 3D scaffolds which were impregned with Ca-phosphate or silica. Investigations using SaOS-2 cells revealed an increased growth and enhanced mineralization of the cells grown onto silica impregnated scaffolds than onto Ca-sulfate grids. Moreover, **UMC-Mainz** developed a novel material based on polyP and a modified chitosan that can be bioprinted and hardened in a controlled way. **UEX** applied different materials, comprising hydroxyapatite, beta-tricalcium phosphate and 45S5 bioglass, for preparation of robocast scaffold samples which were subjected to bio-silica coating by **UMC-Mainz**, using Glu-tag silicatein. Moreover, **UEX** successfully applied a Ca-polyP coating to bioceramic (hydroxyapatite and beta-tricalcium phosphate) robocast scaffolds, in addition to the bio-silica coatings deposited by **UMC-Mainz** onto HAp scaffolds. **UMC-Mainz** developed a bio-inspired method for hardening of bio-silica scaffolds, based on a combined application of b3D bioprinting of matrix-embedded cell units and electrospinning. This partner could demonstrate that a morphogenetically active matrix can be produced using TEOS containing electrospun nanofibers mats, consisting of poly(epsilon-caprolactone), and incubation with silicatein.

In detail:

Design and Fabrication of 3D nanocomposite scaffolds applying three different procedures

a) Robocasting: UEX has developed novel strong and tough ceramic/polymer composite scaffolds by impregnation of bioceramic (HAp, beta-TCP, 45S5 and 13-93 bioglasses) robocast scaffolds with biodegradable synthetic (PLA, PCL) and natural (chitosan, alginate and gelatin) biodegradable polymers. Both fully impregnated and just coated structures have been produced.

UEX extensively worked on the impregnation of 45S5 bioglass scaffolds with natural polymers (alginate, chitosan, and gelatin) and, in collaboration with **BUT**, PCL/HAp nanocomposite coatings to develop hybrid scaffolds exhibiting both enchanced mechanical properties (i.e. superior strength and toughness) and improved biological performace.

The effect of different processing variables (solvent, infiltration temperature, concentration, particle morphology) on the mechanical and *in vitro* biological performance of the new hybrd structures has been analysed, and the characterization and optimization of all the materials developed in previous years have been completed.

Besides, the effect of different geometrical parameters (rod diameter, spacing, layer

overlapping, angle between layers, etc.) of the robocast structures (both bare and fully impregnated) on their mechanical performance is being analysed by finite element (FEM) numerical simulations. Some of the interesting predictions obtained by FEM—for example, it is found that the fracture mode and predicted strength depend significantly on layer overlapping)—has been (and are being) verified experimentally. Samples with different geometrical designs have been prepared to analyse these effects of pore architecture on the mechanical properties (namely strength) of the developed materials with the ultimate aim of optimizing their mechanical performance and enable their usage in load bearing regions of the skeleton.

b) 3D ink-jet printing: UMC-Mainz and **NANOTEC** prepared 3D scaffolds mainly by the layer-by-layer 3D printing method using the ZPrinter 450. These partners focused on the 3D bioprinting method, as an additive manufacturing method.

To determine the growth of SaOS-2 cells onto the scaffold, the cells were seeded to the 24-multi-well plates, into which the grid layers were inserted, and cultivated. Then the cells were removed and the grid layers inspected by SEM. The solid grids both the non-processed Ca-sulfate, and the Ca-phosphate or silica processed grid layers retain their overall structural integrity. The Ca-sulfate grids are colonized with a lower number of SaOS-2 cells, compared to the grids processed with Ca-phosphate or silica.

To investigate the mineralization of SaOS-2 cells onto the scaffold, the OsteoImage dye staining system and DRAQ5 dye for the staining of the nuclei were applied. The samples were analyzed by confocal laser scanning microscopy. The images showed that the cell densities, as assessed by DRAQ5 staining, are only marginally different in the three assay systems (Ca-sulfate, Ca-phosphate, and silica). However, if the intensities obtained after the OsteoImage fluorescence staining were compared it became obvious that the OsteoImage intensity in the assays of SaOS-2 cells, grown on Ca-sulfate, was significantly lower, if compared to the intensities recorded in samples, grown onto Ca-phosphate grids. The highest staining intensities were seen after inspection of the cells, grown and activated onto silica grids.

c) Selective laser sintering: This method has been applied by the Chinese consortium (partner **THU-1**); see below (Summary of the results from Chinese consortium).

Implementation of the micro-units into the rapid prototyping process: UEX has assessed that while the incorporation of the micro-units to the robocasting process is feasible, the process has some important drawbacks to be a practical process for the fabrication of 3D scaffolds. Inhomogeneus micro-unit sizes and a tendency to fracture during the scaffold preparation process (powder dispersion, ink preparation, drying, debinding and sintering) are two of the main hurdles rendering this process impractical.

As an alternative, **UEX** has developed scaffolds with internal channels for nutrient delivery by extrusion of existing bioceramic (HAp, TCP) inks through coaxial nozzles. After optimization of the deposition parameters, scaffolds with a continuous internal channel were achieved. These scaffolds could find potential applications for guided growth of tissue (e.g. nerve guides) or the development of new bioreactors.

Development of a bio-inspired method for hardening of bio-silica scaffolds: UMC-Mainz developed a novel strategy to increase the hardness of the scaffold material, based on the combined application of bioprinted matrix-embedded cell units and electrospun mats. **UMC-Mainz** showed that electrospun poly(epsilon-caprolactone) (PCL) nanofibers mats containing TEOS and subsequently incubated with silicatein, provide a morphogenetically active matrix for the growth and mineralization of osteoblast-related SaOS-2 cells. In addition, **UMC-Mainz** succeeded to incorporate Ca-

polyP nanoparticles into the electrospun fibers. Poly(D,L-lactide) (PLA) was mixed with PEG and stirred for 6 h, until complete dissolution of the polymers was obtained. Ca-polyP nanoparticles were added to the PLA solution. The suspension was sonicated prior to electrospinning to obtain a suitable dispersion of the nanospheres in the PLA solution. Electrospinning was performed with a Spraybase electrospinning unit. A metallic net was used as the collector. The fiber mats were removed from the collector and dried overnight. The electrospun fibers turned out to be morphogenetically active. In addition, **UMC-Mainz** developed a novel material based on polyP and a modified chitosan that can be bioprinted and hardened in a controlled way.

Modification of scaffolds with bio-silica/silicatein or bio-polyP: UEX has completed the characterization of their HAp robocast scaffolds coated with bio-silica by **UMC-Mainz** and a positive effect on *the in vivo* biological performance has been demonstrated, almost as good as that obtained by incorporating rhBMP-2 into the structure. **UEX** has also been able to produce, in collaboration with **BUT**, Ca-polyP coatings onto robocast calcium phosphate scaffolds and demonstrated that such bio-inorganic coatings do not affect negatively the mechanical performance of such scaffolds.

In addition, **UMC-Mainz** developed a method for coating of titanium oxidized Ti-6Al-4V scaffolds with biologically active amorphous Ca-polyP microparticles using the silane coupling agent APTMS. After etching the metal surface was covalently linked with APTMS. The Ca-polyP particles were attached to the surface via Ca^{2+} ionic linkages.

- **Highlights**:

  - **First morphogenetically active 3D nanocomposite scaffolds prepared by 3D printing developed (UMC-Mainz)**

  - **Method for hardening of biosilica-containing scaffolds (UMC-Mainz)**

  - **Combined method using electrospun nanofibers and bioprinted scaffold as a novel strategy to increase hardness of morphogenetically active, cell-containing hydrogel scaffolds (UMC-Mainz)**

  - **Novel material based on polyP and a modified chitosan that can be bioprinted and hardened in a controlled way (UMC-Mainz)**

  - **A new sintering route to produce dense 45S5 scaffolds at lower temperature (550°C) has been discovered (UEX)**

  - **Novel biodegradable ceramic-polymer composites have been developed by deposition of natural polymer and PCL/HAp nanocomposite coatings onto robocast scaffolds (UEX)**

  - **Enhanced mechanical properties and *in vitro* performance of hybrid ceramic/polymer structures have been demonstrated (UEX)**

  - **Scaffolds with continuous internal channel have been produced by coaxial robocasting (UEX)**

  - **Ca-polyP coatings have been deposited onto robocast bioceramic scaffolds without adverse mechanical effect (UEX).**

  - **Feasibility of bio-silica coating deposition on robocasting scaffolds has been demonstrated (UEX)**

- **Enhanced biological performance of bio-silica coated robocast scaffolds has been demonstrated (UEX)**

4. 3D CELL PRINTING AND SUBTRACTIVE MANUFACTURING

Summary:

UMC-Mainz, together with **NANOTEC**, demonstrated that bio-silica-containing alginate hydrogels are a suitable bioprintable and biodegradable matrix for embedding bone-forming SaOS-2 cells. The morphogenetic activity of silica is retained by cells after encapsulation into Na-alginate. In addition, **UMC-Mainz** developed a material that can be bioprinted. This material is composed of a modified chitosan (*N,O*-CMC), polyP and alginate. This material can be hardened, after printing, by exposure to calcium ions. In addition, **UMC-Mainz** and **NANOTEC** prepared bioprintable cell-containing scaffolds, containing bioglass encapsulated into a with calcium-chloride-hardened alginate/gelatine hydrogel; the hydrogel supplemented with polyP•Ca^{2+} or enzymatically – *via* silicatein – prepared biosilica was found to promote the growth and mineralization of the SaOS-2 cells. **BUT** fabricated 3D nanocomposite scaffolds using 3D milling of gelcast hydroxyapatite foams and optimized scaffold fabrication from the viewpoint of higher milling accuracy and developed a method for modification of the ridigid ceramic scaffold by calcium polyphosphate (polyP Ca^{2+} salt) coating. This coating approach was also applied to bioglass and titanium scaffolds prepared by SFF methods. Ceramic foams with different structures and mechanical properties were prepared and characterized. In addition, **BUT** prepared prototypes of scaffolds for a model defect in human jaw and for lumbar intervertebral fusion in pigs. **UEX** succeeded to prepare SFF robocasting preforms suitable for 3D milling and required manufacturing processes to fabricate prototype customized scaffolds. Small series of components for evaluation by the clinical partners were fabricated (**UMC-Mainz** and **NANOTEC**). **NANOTEC** designed/developed further applications of the new techniques in orthopedics and dentistry, e.g. for the sealing of teeth.

In detail:

3D printing of cells embedded in bio-silica alginate hydrogel: UMC-Mainz, together with **NANOTEC**, embedded SaOS-2 cells into Na-alginate, supplemented with biosilica. It was found that the morphogenetic activity of silica is retained by SaOS-2 cells that have been encapsulated into Na-alginate. Based on the finding that Na-alginate is a suitable matrix for embedding bone cells **UMC-Mainz** successfully started to print 3D structures in order to apply this technology for bioprinting and construction of bioartificial tissues or organs. In a first step **UMC-Mainz** encapsulated separately bone-forming (SaOS-2) and bone-degrading (RAW 264.7) cells to develop a biomimetic synthetic scaffold suitable for tissue engineering. In the alginate matrix applied the SaOS-2 cells retain their capacity to synthesize HA crystallites. Furthermore, the mechanical properties, including surface roughness and hardness, of the hydrogel were determined. If silica is included in the hydrogel matrix, the encapsulated SaOS-2 cells were found to increasingly express the gene encoding for osteoprotegerin in co-cultivation experiments with RAW 264.7 cell beads, suggesting that under the applied conditions the differentiation capacity of the RAW 264.7 cells is impaired. In continuation it was found that under these conditions (SaOS-2 cells cultured together with RAW 264.7 cells) the RAW 264.7 cells show a reduced capacity to express the gene for tartrate-resistant acid phosphatase.

UMC-Mainz further applied this knowledge to form with Na-alginate, enriched with bicarbonate and or biosilica, a matrix suitable for bioprinting. For rapid prototyping

bioprinting **UMC-Mainz** used a computer-aided tissue engineering printer (3D-Bioplotter; Corporate EnvisionTEC GmbH). With this technology **UMC-Mainz** succeeded to embed SaOS-2 cells into the Na-alginate, with the indicated supplements, and allowed the matrix to be passed through the capillary of the 3D printer; 4 mm high blocks were printed into which the cells remained viable and retained the capacity to form mineralic crystallites.

In a new approach for preparing bioprintable cell-containing scaffolds, **UMC-Mainz** and **NANOTEC** investigated the effect of bioglass which is not suitable for embedding bone cells, on growth and mineralization of bone-related SaOS-2 cells encapsulated into a printable and biodegradable alginate/gelatine hydrogel hardened with calcium chloride. The hydrogel was supplemented either with polyP, given as polyP•Ca^{2+}-complex, or biosilica, enzymatically prepared from ortho-silicate by silicatein.

The results revealed that solid bioglass (nano)particles, with a size of 55 nm and a molar ratio of SiO_2:CaO:P_2O_5 of 55:40:5, if added to the cell-containing alginate/gelatin hydrogel, supplemented with either polyP•Ca^{2+}-complex, biosilica, or polyP•Ca^{2+}-complex *and* biosilica, did not change the growth of the cells. SEM analyses revealed that the samples containing only alginate/gelatin show an almost homogeneous appearance. Addition of silicatein to the gel does not change the homogeneous pattern. However, if ortho-silicate is added to the hydrogel the samples comprise clusters of 500 nm-sized silica drops. The clusters and patches of biosilica within the hydrogel are widespread. Addition of polyP•Ca^{2+}-complex to the hydrogel, prior to the bioprinting process, revealed the appearance of crystal-like precipitates in the hydrogel cylinders. If the hydrogel is supplemented both with polyP•Ca^{2+}-complex and biosilica the characteristic deposits, crystal-like for polyP•Ca^{2+}-complex and round-shaped clusters of drops for biosilica become obvious.

In addition, **UMC-Mainz**, together with **NANOTEC**, developed polyP-based material that can be bioprinted to tissue-like units with a controlled morphology by complexing polyP (morphogenetically active polymer) with chitosan and alginate (bioinert polymers). Chitosan cannot form a complex with polyP. Therefore, **UMC-Mainz** derivatized chitosan to *N,O*-carboxymethyl chitosan (*N,O*-CMC). Thus, the resulting novel bioprintable material is composed of three polyanionic polymers: alginate, *N,O*-CMC and polyP. After printing the material is exposed to Ca^{2+} in order to harden the material and to make it more durable. During Ca^{2+} exposure the Na^{+} cations in polyP are exchanged by Ca^{2+} allowing the bridging of polyP to *N,O*-CMC and rendering this composite material to a persistent structure and morphology, without losing the biological activity of polyP. The organization of this polyP-*N,O*-CMC composite material to durable tissue-like moldable blocks has been achieved by alginate. This polymer can form, *via* electrostatic forces between the carboxyl groups within the alginate and the counterion species (e.g. Ca^{2+}), gels of a controlled strength and porosity. Based on these properties **UMC-Mainz** described a formula for a matrix (*N,O*-CMC, polyP and alginate) that can be bioprinted as soft, moldable 3D structures. Those 3D printed molds are, in the subsequent fabrication stage, hardened by exposure to Ca^{2+}.

By using the layer-by-layer printing approach, several implants fitting to defects in the underjaw, after having analyzed the lesions by µCT, have been fabricated from the N,O-CMC+polyP scaffold and hardened by Ca^{2+}.

Design and fabrication of 3D nanocomposite scaffolds applying 3D milling of gelcast foams and SFF preforms: The process for fabrication of hydroxyapatite scaffolds by 3D milling of gelcast foam has been optimized. **BUT** demonstrated the complete fabrication process from suspension preparation through foam consolidation to 3D milling of a scaffold. The milling process of a scaffold has been optimized from the viewpoint of obtaining precise scaffold dimensions and complex shape reproduction

of the digital model. The foam consolidated using a new gelling agent (epoxy resin) provided porous foam (blank) that can be easily milled with high accuracy and even the tiny details and sharp edges could be produced with high precision. The surface and edge quality was comparable to or even better than the surface quality of the SFF scaffolds.

Modification of scaffolds with bio-silica/silicatein or bio-polyP: BUT has developed a coating process that can deposit a few microns thick layer of calcium polyphosphate (polyP Ca^{2+} salt) on a surface of 3D milled scaffolds. This two-step process is based on coating the scaffold with soluble sodium polyphosphate, which is in the second step converted to insoluble gel-like layer of calcium polyphosphate. The process is applicable to complex scaffolds even with microporous surface. The layer of calcium polyphosphate firmly adheres to the surface and can be easily handle with the scaffold in the dried state. The coating process was successfully applied also to bioglass and titanium scaffolds.

Manufacturing processes of the ceramic and bioinorganic polymer materials and prototype development: BUT successfully demonstrated the applicability of the developed manufacturing process based on 3D milling of modified ceramic foam by preparing prototypes of scaffolds for a model defect in a human jaw and for a lumbar intervertebral fusion in pigs.

UEX developed and optimized robocasting ink compositions (from graphite and polymers) suitable to be used as sacrificial support materials for the fabrication of complex structures, as well as their respective burn-out treatments. The procedures for the automatic printing of complex structures using two tips were also developed and optimized. All these optimized processes were used to successfully produce prototype calcium phosphate robocast scaffolds that fit into an artificially created bone defect on a human mandible.

UMC-Mainz fabricated small series of components for bioprinting scaffolds for evaluation. PolyP microspheres with different ranges of diameters were fabricated. The surface of the microspheres had a porous texture with a pre size of 25-30 nm. The hardness (Young's modulus) of the particles was determined with a nanoindenter.

Development of applications of the developed techniques in orthopedics and dentistry: NANOTEC designed/developed new applications of the developed techniques in orthopedics and dentistry. The SME partner demonstrated that the fabricated amorphous Ca-polyP microparticles (aCa-polyP-MP) efficiently reseal enamel defects. Electron microscopical and EDX studies showed that the Ca-polyP particles form a nearly homogenous, 50-µm thick polyP layer on the tooth cement and dentin surfaces.

- **Highlights**:

  - **Development of the first morphogenetically active cell-containing hydrogels for 3D bioprinting (UMC-Mainz)**

  - **Proof-of-concept of 3D printing of cells embedded in bio-silica or polyP-containing alginate hydrogel (UMC-Mainz)**

  - **Novel matrix consisting of N,O-CMC, polyP and alginate, that can be bioprinted as soft, moldable 3D structures and subsequently hardened by exposure to calcium ions (UMC-Mainz)**

  - **Development of new applications of the developed techniques in**

orthopedics and dentistry (NANOTEC and UMC-Mainz)

- **Fabrication of small series of components for evaluation by the clinical partner – microparticles/microspheres (NANOTEC and UMC-Mainz)**

- **Development of the process for fabrication of ceramic scaffolds by precise 3D milling of ceramic foam (BUT)**

- **Modification of rigid ceramic scaffolds by coating the surface with calcium polyphosphate (BUT)**

- **Fabrication of scaffold prototypes by 3D milling of porous foam (BUT)**

5. CHARACTERIZATION AND CLINICAL TESTS

Summary:

The partners characterized the microstructural, mechanical and biological properties of the newly developed scaffolds and scaffold materials. Determination of the material properties of the micro-units by **BUT** revealed, using the applied processing conditions, micro-units with the optimal outer diameter and inner (microchannel) diameters can be formed, as well as thin-wall micro-tubes by using slow dip-coating rates. Scanning electron microscopy, as well as mercury and water porosimetry, was applied to characterize the hydroxyapatite foams. In addition, the mechanical behaviour of the ceramic foams were characterized. **UEX** completed the microstructural and mechanical characterisation of all the bioglass-polymer composites developed. Moreover, the in vitro degradation of such materials upon inmersion in SBF was also analysed for all compositions. Some *in vitro* and *in vivo* biological characterization was also performed in selected compositions, including in biosilica-coated hydroxyapatite scaffolds. The determination of the structural and mechanical properties of the of the 3D bioprinted (*N,O*-CMC/Na-polyP/Na-alginate) hydrogel scaffolds was performed by **UMC-Mainz** and **NANOTEC**. Various compression tests were applied to determine the local (nanoindentation) and bulk mechanical properties (tensile/compression test system for force measurements) of the *N,O*-CMC-polyP-alginate material. The investigation of the effect of the scaffolds on adhesion, proliferation, HA formation and expression of growth factors / cytokines of osteoblast-like cells by **UMC-Mainz** revealed an increased expression of *BMP-2* and *OPG* (as well as an increased expression ratio *OPG*:*RANKL*; determined by qRT-PCR). Addition of bioglass into a printable and biodegradable alginate/gelatine hydrogel containing encapsulated SaOS-2 cells caused an enhanced mineralization by the cells. Similar results were found for SaOS-2 cells growing on the electrospun PCL nanofiber mats after a pre-incubation of the fibers with silicatein in the presence of a suitable silica precursor. The effect of the scaffolds on growth and differentiation of human adipose-derived stem cells into osteoblasts is performed by the Chinese partner **PKU**, as well as part of the animal tests (nude mice and rabbits). First animal experiments (rabbit patellar groove model for cancellous bone healing) of the newly developed morphogenetically active materials, performed by **UMC-Mainz** in cooperation with **NANOTEC,** using PLGA microspheres containing encapsulated silicatein and silica revealed an enhanced regeneration of bone tissue, compared to the control implants containing beta-TCP or silicatein only. Further animal experiments by **UMC-Mainz** in cooperation with **NANOTEC** were performed using the rat calvarial defect model with the newly developed morphogenetically active biosilica-based or polyP-based materials.

In detail:

Characterisation of the material properties and of the mechanical properties: The micro-units were characterized by **BUT** by means of scanning electron microscopy. The results of these observations showed that by optimization of processing conditions the forming of micro-units with desired outer diameter (ca 50 µm) and inner microchannel is possible. Additionally, using slow dip-coating rates, **BUT** was are able to prepare also the thin-wall micro-tubes. The sintered micro-units were characterized in terms of mean grain size and porosity of the samples.

The HA foams were characterized by means of scanning electron microscopy (morphology of the pore structure, grain size), mercury and water porosimetry (pore size, total porosity, pore interconnectivity, and pore size distribution).

The most suitable foam structure from the viewpoint of biological applications was prepared from a suspension with 32.5 vol% powder loading and had ceramic cells in the range from 300 to 1100 µm with a mode at 650 µm with connecting windows between the cells in a range from 100 to 350 µm. This foam also exhibited the most uniform compression strength with an average value of 2.0 MPa. Such a compressive strength together with a high foam porosity of over 83% present excellent scaffold material characteristics. Such scaffolds enable safe handling and shape forming of bone defects with a minimum amount of the scaffold material in the bone defect that remains or has to be resorbed during bone regeneration.

The developed hybrid ceramic/polymer structures, including the new natural polymer-coated composites and scaffolds reinforced by composite coatings, have been microestructurally and mechanically characterized by **UEX** both in compression and in bending. The analysis of the results obtained has provided a more profound understanding of the parameters affecting the mechanical properties enhancement provided by polymer impregnation, which have important implications for the effective use of this strategy to improve the mechanical performance of robocast scaffolds.

Besides, pure bioceramic structures fabricated by robocast with dense struts such as those developed by using 13-93 bioactive glass can exhibit strength values in the cortical bone range while keeping porosities around 50% with a high degree of pore interconnectivity. Another interesting results regarding this later material, is that we have demonstrated that the microporosity within the rods of 13-93 robocast scaffolds determines not only the initial strength of the structures but also their degradation rate and maximum percentage of degradation in vitro. This paves the way for controlling the degradation behaviour of this material in order to match it to the rate of bone formation, with the aim of assuring the mechanical integrity of the implant/tissue system for the duration of the regeneration process.

Finally, regarding the optimization of the geometrical parameters defining the pore architecture of robocast structures, the experimental tests carried out have confirmed the finite element predictions, including the existence of a new, as yet unreported, fracture mode under compression for brittle robocast structures with low layer overlapping, which was confirmed by in-situ observations carried out during the tests. The results have enabled us to derive an important conclusion for the mechanical optimization of robocast structures: a layer overlapping of around (but not lower than) 20% allows us to maximize porosity without compromising dramatically the strength of the structure. This is shown here just as an example of similar design guidelines that are being extracted from the variation of other geometrical parameters in studies still underway, not only on bare structures but also on fully impregnated systems.

The results from natural polymer coatings onto 45S5 robocast scaffolds has evidenced that even although such polymers are typically weaker that synthetic alternatives, their stronger interfacial adhesion to the bioceramic substrates makes them excellent

candidates as reinforcement agents. Scaffolds coated with natural polymer exhibit significantly enhanced strength and toughness when compared with synthetic polymers deposited using solutions with the same concentration.

On the other hand, the characterization of *in vitro* degradation of these composite scaffolds has shown that the polymer coatings can be used to adjust the degradation rate of the robocast scaffolds. The higher degradability of the natural polymers enables even to enhance the degradation rate in some cases, although the general effect is to retard the degradation kinetics of the bioceramic or, in this case, bioglass substrate. Moreover, natural polymers can enhance the rate of conversion to calcium phosphate of the bioglass. This is especially true in the case of chitosan coatings which are also the ones yielding the greater strengthening and toughening, which makes this polymer an extremely atreactive candidate as reinforcing agent for bioceramic scaffolds.

Further mechanical enhancement is possible also thorugh the deposition of composite coatings, as demonstrated in collaboration with **BUT**, by depositing PCL coatings loaded with HAP particles onto 45S5 bioglass robocast scaffolds.

UMC-Mainz and **NANOTEC** investigated the structural and mechanical properties of the of the 3D bioprinted hydrogel scaffolds, before and after implantation after various time intervals. **UMC-Mainz** also performed nanoindentation experiments with a biocompatible bioscaffold material, composed of polyP. Poly(D,L-lactide-co-glycolide) (PLGA) microspheres with a narrow size distribution (about 800 µm) were prepared, containing either encapsulated polyP or beta-tricalcium phosphate (beta-TCP), used as a reference material. Discs with a diameter of 8 mm were prepared from the microspheres and inserted into 10 mm large defects created in the calvaria of rats and the kinetics of bone regeneration was evaluated. Nanoindentation experiments showed that during a 56 d healing period the hardness/stiffness (reduced Young's modulus) of the tissue around the implanted polyP spheres reached values of about 2 MPa which are nearly the same as those found in control calvarial bone, whereas the hardness around the control and beta-TCP spheres was much lower (<1 MPa).

Characterization of the physiological properties: To determine the physiological properties of the silica impregnated 3D printed scaffolds fabricated by **UMC-Mainz** and **NANOTEC** using the ZPrinter 450, the grids were submerged in HEPES buffer prior to the cell culture experiments. The expression levels of *OPG* and *RANKL* were determined by qRT-PCR. The data show that the transcript level of *RANKL* does not significantly change from day 1 to day 5. However, the expression of *OPG* increases significantly from 8.9-fold with respect to GAPD steady-state level (day 1 after addition of the mineralization cocktail) to 31-fold at day 5. In turn, the expression ratio between *OPG* and *RANKL* increases as well from 1.6-fold (day 1) to 4.7-fold (day 5).

To determine the induction of *BMP-2* transcription in SaOS-2 cells growing onto silica scaffold, SaOS-2 cells were incubated in mineralization medium onto Ca-phosphate or silica impregnated Ca-sulfate scaffold. The data show that the steady-state transcript level of BMP-2 does not significantly change in SaOS-2 cells after incubation on Ca-phosphate impregnated scaffold. In contrast, if the cells were seeded onto silica-treated scaffold a significant increase of the transcript level is seen already after 3 d.

The experiments with SaOS-2 cells encapsulated into a printable and biodegradable alginate/gelatine hydrogel revealed that addition of bioglass to the hydrogel, in the Alizarin Red S assay, significantly enhanced the increase in mineralization caused by these additives, compared to assays without bioglass. It was found that bioglass added to the hydrogel increased the proliferation and mineralization of bioprinted SaOS-2 cells. The results show that the development of cell-containing scaffolds consisting of a bioprintable, solid and cell-compatible inner matrix surrounded by a printable hard and

flexible outer matrix containing solid bioglass provide a suitable strategy for the fabrication of morphogenetically active and biodegradable implants.

In addition, **UMC-Mainz** characterized the tissue-like-blocks, formed by *N,O*-CMC and Na-polyP in Na-alginate and linked together *via* Ca^{2+} ionic bridges. The *N,O-CMC+polyP* scaffold had a stiffness (reduced Young's modulus [RedYM]) of 935 kPa, while the RedYM of the scaffold lacking polyP (*N,O*-CMC) was only 27 kPa. The reduced modulus for trabecular bone from rabbit amounted to 2,300 kPa. After submersion of the *N,O-CMC+polyP* scaffold in simulated body fluid, no significant change of the RedYM stiffness was observed during a period of 3 weeks (the values determined were around 900 kPa). The decrease of the RedYM became significant (reduction to 686 kPa) only after a period of 6 weeks.

Cell culture experiments revealed that the *N,O-CMC+polyP* scaffold, consisting of polyP bound to *N,O*-CMC, caused an induction of the mineralization of the cells that was significantly higher than the mineralization determined for the *N,O*-CMC scaffold, lacking polyP.

***In vivo* animal tests:**

UMC-Mainz, in cooperation with **NANOTEC**, evaluated the developed morphogenetically active materials in animal experiments. The first animal experiments were performed in rabbits (patellar groove model for cancellous bone healing. Beads (microspheres) containing encapsulated silicatein and silica were tested. The beads (microspheres; diameter about 800 µm) were prepared by encapsulation of beta-TCP, either alone (control) or supplemented with silica or silicatein, into the biodegradable copolymer poly(D,L-lactide-co-glycolide) (PLGA); 5% beta-TCP, 9% silica, and 0.32 µg/mg of silicatein were entrapped into the PLGA microspheres.

The microspheres were implanted into 5 mm thick holes that were drilled into the femur of the animals. A bilateral comparison study design with 3 test groups of 4-8 animals each was applied: The control implant on one of the two hind legs contained microspheres with only beta-TCP, while the test implant on the corresponding leg consisted either of microspheres containing beta-TCP and silica, or a 1:1 mixture of microspheres, supplemented with beta-TCP and silica, and beta-TCP and silicatein.

The results revealed that tissue/bone sections of silica containing implants and implants, composed of a 1:1 mixture of silica-containing microspheres and silicatein-containing microspheres show an enhanced regeneration of bone tissue around the microspheres, compared to the control implants containing only beta-TCP. The latter material caused a much stronger lamellar bony apposition from the interface of the implanted beads and a stronger bone growth in the cancellous bone trabeculae around the interface as compared to beta-TCP and silica.

The process of regeneration in the bone regions that harbored the silica-containing implants (silica-containing microspheres and silicatein-containing microspheres) was substantially more distinct compared to the control implants containing only beta-TCP. The stiffness (reduced Young’s modulus) was 1,450 kPa around the implants in the regenerating regions, while the stiffness for the control microspheres with beta-TCP was only 430 kPa. In the vicinity of the microspheres containing only silicate (without silicatein), the stiffness was only 1,130 kPa. For comparison, the values for the stiffness of the trabecular bone tissue and for the tissue/cells in the hole of the implants was 2.3 MPa and 70 kPa, respectively.

In the second series of animal experiments, **UMC-Mainz**, in cooperation with **NANOTEC**, tested the polyP scaffold, after embedding into beads. The effect on bone

regeneration was studied in the rat calvarial defect model. A 10 mm large defect was set in the dorsal surface of the cranium over the calvarium. After setting a 10 mm large defect one implant disc each per animal was inserted with a diameter of 8 mm; the discs had been assembled from 800 µm sized microspheres. After surgery and care of the laboratory animals they were kept for 28 d or 56 d under strictly controlled husbandry conditions. Three animal groups were formed, each containing 4 experimental animals. As a control, without active supplements discs with “micro” beads were used. As an introduced osteoconductive and biodegradable mineralic implant material we have selected beta-TCP and fabricated this synthetic bone grafting material into the PLGA scaffold “beta-TCP-micro”. polyP was entrapped into the PLGA under formation of the “polyP-micro” discs.

The process of mineralization was determined after sectioning the implant zone by applying the histochemical Masson's trichrome staining procedure. The healing zones were removed from the animals after 28 d or 56 d. It was found that after a healing period of 28 d, areas that are stained in green and that reflect mineralizing collagen rich areas are very intense in the samples with the “polyP-micro”, compared to the controls and the “beta-TCP-micro”. The areas stained in green increase in size after an implantation period of 56 d. The progression of the mineralization in samples that received polyP-containing microspheres show an almost homogeneous regeneration and the infiltrating tissue becomes functionally connected with the surrounding cell layers.

In addition, **UMC-Mainz** applied the load-displacement measurements/ nanoindentation, based on a cantilever on the top of a glass ferrule that hosts an optical fiber for readout, to quantitate the elastic modulus and hardness in the regions between the microspheres. The moduli of the mineralized areas around the spheres within the regenerating regions vary significantly. The lowest values are found around the control “*micro*” beads with 153 kPa. Significantly higher are the moduli around the implanted “*beta-TCP-micro*” and “*polyP-micro*” beads with 484 kPa and 2,010 kPa, respectively. The data confirm the results outlined in the previous section.

Moreover, animal experiments were performed with *N,O*-carboxymethyl chitosan (*N,O*-CMC), that mimics the natural extracellular matrix, and has been provided with functional activity by integration of polyP. The two polymers, *N,O*-CMC and polyP, were linked together via Ca^{2+} bridges. This material was proven to be printable and durable. Animal experiments revealed a strong regeneration-inducing activity of the material in the rat calvarial defect model. In turn, *N,O*-CMC + polyP represents a promising hybrid material for the fabrication of customized scaffolds.

Finally, **UEX** and **UMC-Mainz** with the aid of external collaborators from Universidad Complutense de Madrid, were able to evaluate *in vitro* e *in vivo* the biological performance of biosilica-coated HA scaffolds fabricated by robocasting. For the *in vivo* tests, disc-shaped robocast scaffolds were implanted in rabbit crania and the performance of both coated and uncoated scaffolds was evaluated with and without the addition of rhBMP-2. Biosilica coated scaffolds exhibited improved osteointegration compared to uncoated HA. The biosilica sample present an increase of new bone tissue inside of its structure where it is possible to detect at higher magnification a dense collagen matrix, osteoblastic cells dispersed on the tissue and an increased number of blood vessels, confirming that angiogenesis is taking place. The best results were obtained when combining both approaches (bio-silica + rhBMP-2).

Based on the results of the animal eperiments, our new materials turned out to be:

- Biocompatible and biodegradable
- superior to beta-TCP and HA in supporting bone healing

- **Highlights**:

  - **Structure and pore size distribution of ceramic foams described (BUT)**

  - **Mechanical behaviour of ceramic foams in compression determined (BUT)**

  - **Hybrid ceramic/polymer structures developed in Task 3.1, especially 13-93/PCL composites, can match or even surpass cortical bone mechanical performance in terms of strength and toughness (UEX).**

  - **Robocast 13-93 bioactive glass scaffolds exhibit cortical bone strengths and their degradation rates can be controlled by the level of in-rod microporosity (UEX)**

  - **Geometrical study is enabling the optimization of pore architectures in robocast scaffolds (UEX)**

  - **Coating with natural polymers, especially chitosan, enhances simultaneously the mechanical and biological performance of 45S5 bioglass robocast scaffolds (UEX)**

  - **Composite coatings enhance the strength of robocast scaffolds even further than the pure polymers, especially when the reinforcing particles are small and non-agglomerated (UEX)**

  - ***In vitro* degradation of bioceramic and bioglass scaffolds can be modulated by polymer impregnation (UEX)**

  - **Biosilica coatings enhance *in vivo* performance of HAp robocast scaffolds and its effect is compatible/enhanced by the incorporation of rhBMP-2 (UEX)**

  - **Advantageous mechanical properties of the new biosilica- and polyP-containing scaffold materials and cell-containing hydrogels demonstrated (UMC-Mainz)**

  - **Enhanced hydroxyapatite formation on the newly developed 3D printed scaffolds demonstrated (UMC-Mainz)**

  - **Demonstration/proof-of-concept of morphogenetic activity of cells growing on the newly developed 3D printed scaffolds (UMC-Mainz)**

  - **Increased mechanical stability of bioprinted alginate - cell hydrogel units by enwrapping in biosilica-containing electrospun nanofibers mats (UMC-Mainz)**

  - **Successful bioprinting and demonstration of the mineralization promoting activity of the newly developed *N,O-CMC+polyP* scaffold (UMC-Mainz)**

  - **Demonstration of the adaptable hardness of the *N,O-CMC+polyP* scaffold with respect to the healing process using a new indenter device with a cantilever on the top of a glass ferrule (UMC-Mainz)**

  - **Successful first animal experiments using beads (microspheres)**

containing encapsulated silicatein and silica for treatment of bone defects (rabbit patellar groove model for cancellous bone healing) (UMC-Mainz and NANOTEC)

- **Successful animal experiments with the new polyphosphate-containing scaffold (rat calvarial defect model) (UMC-Mainz)**

6. INTELLECTUAL PROPERTY AND TECHNOLOGY TRANSFER

Summary:

The SME partners involved in this project forming the SME & IP Board (**ORTOMA** and **NANOTEC**) were responsible for the management of the knowledge portfolio available in the consortium. New PCT applications related to the Bio-Scaffolds project are in preparation and will be submitted by consortium members. In addition, the SME partners searched for potential user groups and established new contacts with other companies in the target market (scaffold materials; medical implants). They also closed first cooperation agreements with larger industrial companies.

Integrated management of knowledge and intellectual property: New products developed in the project were always analyzed for their potential patentability. These activities were supported by the SME & IP Board which was in charge of the analysis of the technical expertise of the partners and the identification of potential inventions. This board also supported the partners in the establishment of IP-related contacts with external partners from industry.

Building-up a network structure: The patent portfolios of the Bio-Scaffolds consortium members have been analysed by the SME & IP Board. Several partners are already owners of a number of granted patents or patent applications, in which the partners from academia are inventors. These partners gave advice to the other partners, or contributed by giving presentations about IP relevant issues during each project meetings/workshops.

Filing of patents and granting licenses: A number of new patent applications hat are based on the results of the project is in preparation. **ORTOMA** has filed three patent applications during the period for planning and system functionality.

Technology transfer: The SME partners involved in this project (European partners **ORTOMA** and **NANOTEC** and Chinese partner **LANDO**) had intense contacts with other industrial companies as well as technology transfer companies. These contacts already resulted in the conclusion of cooperation agreements with larger industrial partners. The target markets are: New regeneratively active scaffold materials that can be used for the customized fabrication of medical implants. Several cooperation agreements have been concluded. **ORTOMA** is currently in negotiation with three large university hospitals in Sweden for usage of the system for patient treatment.

- **Highlights**:

  - **Identification of new patent relevant results obtained in the frame of the project (NANOTEC and UMC-Mainz)**

  - **New patent preparations have been prepared or are in preparation (several partners)**

  - **Negotiations to establish a company commercializing robocast scaffolds**

in China initiated, conversations with other industrial networking partners underway (UEX)

Summary of the results from Chinese consortium:

1. IMAGE PROCESSING AND MODELING (THU1)

The objective of the project of **THU-1** was to build personalized 3D model of human bone structure from different modal medical images (CT\MRI\μCT) of different resolutions, thus achieving segmentation of bone and its surrounding tissues, arbitrary cutting of 3D model, extraction of defect tissues, etc. An image processing and modeling software was preliminarily implemented to produce model data. Over 20 groups of CT, over 20 groups of MR and over 10 groups of μCT images were obtained from several hospitals to test the above whole image processing and 3D modeling procedure.

An integrated image processing protocol was developed for the extraction of human anatomical structures based on multimodal medical images. The generated model can be formatted as standard data for 3D rapid prototyping system to manufacture entity stent. It can be also sent as the formats are acceptable by current computer assistant design (CAD) software for modification and editing.

Three 3D modeling manipulation techniques (direct image based modeling; the mirror model to construct defect; and image modeling joint with CAD process) were used for the defect repair. The direct technique could build 3D model of each bone segment without defect directly using the segmented images. The mirror technique could repair the bone defect according to body symmetry. The joint technique can repair the defect with the help of CAD method in case of the above two techniques could not handle. Bone μCT was scanned to build micro-structure 3D model, which described fine structures of bone trabecula.

A CT template and MRI integration method was designed to extract the bone density distribution and the adherent surrounding tissues, in addition to the accurate bone shape modeling. The bone shape was firstly modeled from CT, and was then used as the template to extract bone density. The template was also adapted to extract surrounding tissues from MRI. Finally the bone density field and surrounding tissues was integrated to construct the voxel model, which described the complete information of the bone.

The modeling method was tested and a modeling software was developed, which could output the model for use in several rapid prototyping systems. The generated models were successfully tested on different rapid prototyping technologies by the Chinese and European partners.

2. SELECTIVE LASER MELTING OF TITANIUM ALLOY AND DEVELOPMENT OF HIERARCHICAL SURFACES ON TITANIUM SURFACE (THU-2)

Selective laser melting of titanium alloy and its post heat treatment: The microstructure of as-fabricated Ti-6Al-4V consisted of fine acicular martensite alpha and columnar prior beta grains. The ductility of as-fabricated Ti-6Al-4V falls far short of the requirements for customized titanium alloy scaffolds. Three strategies of heat treatment were proposed to improve of the mechanical properties based on different phase transformation mechanisms and classified as substransus treatment, supersolvus treatment and mixed treatment. The substransus treatment leaded to a basket-weave structure without changing the morphology of columnar prior beta grains.

The supersolvus treatment resulted in a lamellar structure and equiaxed beta grains. The mixed treatment yielded a microstructure that combines both features of the substrasus treatment and supersolvus treatment. The substrasus treatment is found to be the best choice among these three strategies for as-fabricated Ti-6Al-4V to be used as biomedical scaffolds.

Development of hierarchical surfaces on titanium surface: Surface micron/nano-topographical modifications have attracted a great deal of attention because it is capable of mimicking the hierarchical characteristics of bone. In this study, TiO_2/silicate hierarchical coatings with various nanostructure morphologies were successfully prepared on titanium substrates through micro-arc oxidation (MAO) and subsequent hydrothermal treatment (HT). The novel TiO_2/silicate hierarchical coatings comprised calcium silicate hydrate (CSH) as an outer-layer and TiO_2 matrix as an inner-layer. According to the morphological features, the nanostructures were classified as nanorod, nanoplate and nanoleaf.

The TiO_2/CSH hierarchical coatings exhibited some enhanced physical and biological performances compared to MAO-fabricated coating. The improvement of the hydrophilicity, fibronectin adsorption and apatite-inducing ability was found to be morphological dependent according to the following trend: nanoleaf coating > nanoplate coating > nanorod coating > MAO coating. The response of osteoblast like cells (SaOS-2) was studied on each surface after sputtering with a thin layer of gold (Au) to minimize the influence of surface chemistry. The adherent cells were polygonal-shaped on microcrater surface, roundish on nanoplate surface and elongated on nanoleaf surface. Additionally, compared to microcrater surface, nanoplate surface slowed down cell proliferation and exhibited no enhancement on cell differentiation. However, nanoleaf surface supported cell proliferation and promoted cell differentiation. The results indicate that tuning morphological features of nanostructures on micro-topography can serve as a promising strategy to specifically modulate cellular response, such as cell morphology, proliferation, differentiation and mineralization.

***In vitro* cellular response of SaOS-2 cells on the macro/mesoporous TiO_2 coating:** Hierarchical structures in both micro- and nano- scale show synergistic effect on promoting osseointegration between bone and scaffolds. Various surface modification methods have been used to produce hierarchical topography on the titanium scaffolds. Macro/mesoporous TiO_2 coating is generated by combining micro-arc oxidation and evaporation-induced self-assembly methods. The mesoporous film has a pore size of 10 nm and consists of anatase TiO_2 nanocrystallites. The hierarchical macro/mesoporous surface has better hydrophilicity and fibronectin adsorption ability than the macroporous TiO_2 surface and smooth substrate surface. With the formation of nanostructure mesoporous film on macroporous TiO_2 surface, the attached cell number increases after incubation for 4 hours but have no significant differences at 24 hours compared to the substrate surface and macroporous surface. Cells have larger spread areas and more well organized stress fibres on the macro/mesoporous surface than cells on the other surfaces. ROCK inhibitor Y27632 is used to investigate the effect of RhoA/ROCK pathway on the initial cell adhesion on the macro/mesoporous surface. In the presence of Y27632, cells on macroporous surface and macro/mesoporous surface both show stellate appearances, with poor assembly stress fibers and long protusions. Cells on the substrate surface have larger spread areas compared to the former two surfaces. The attached cells significantly reduced treated with Y27632 but there are no differences among the three surfaces. The different cell responses indicate that macro/mesoporous TiO_2 can enhance initial cell adhesion through the RhoA/ROCK pathway. Proliferation of SaOS-2 cells on the macro/mesoporous surface has no significant promotion compared to the macroporous surface but is higher than that on the substrate surface. ALP expression and collagen secretion of SaOS-2 cells are increased on the macro/mesoporous surface, which

indicates better osteogenesis of the hierarchical macro/mesoporous structure. These results suggested that the macro/mesoporous TiO_2 coating is suitable for the scaffold surface.

3. OSTEOGENESIS PROMOTING EFFECT OF THE BIOACTIVE SCAFFOLDS AND THE INFLUENCE OF THE MINERALIZATION ABILITY/ GROWTH BEHAVIOR ON THE HUMAN DENTAL PULP CELLS (PKU)

Osteogenesis promoting effect of the bioactive scaffolds *in vivo* animal test was performed. The test animals are 8-month male beagle dogs. The bio-scaffolds were implanted on the lower jaws of the dogs, after the barbitone intravenous anesthesia. The 2 bone defects of 3x4x5 mm were cut on both sides of the lower jaws, and 4 bio-scaffolds were implanted in each dog. The bone defects and the scaffolds were covered by Collagen membranes, and then the mucoperiosteum was sewn tightly. Specimens were extirpated at 4, 8, and 12 weeks post grafting after sacrificing the animals. Animals were sacrificed by CO_2 asphyxiation. The bone specimens were carefully separated and fixed in 40% formaldehyde solution, and embedded in paraffin. Milling slices of hard tissues are prepared, and stained with toluidine blue, then observed by micrograph to assess the morphology of new bone tissue and osteogenesis. The degradation performance of the scaffold materials was also assessed. The section surface of the bone/scaffolds samples are performed by cross-section polishing and observed by SEM for the details of interface between the bone and materials. The degradation of scaffolds was evaluated by EDS.

The results showed that the bio-scaffolds implanting accelerate the formation of new bone in the bone defect areas. Hydroxyapatite scaffold is degraded during the process of new bone formation and is replaced by the new bone tissue. The bio-scaffolds material is beneficial for osteogenesis, compared with the blank control group.

4. PREPARATION OF PLA/HA BASED SCAFFOLDS AND BETA**-TCP BONE REPAIR MATERIALS BY 3D PRINTING (LANDO)**

The PLA/HA based scaffolds added with multiple release growth factor (P24) microspheres were prepared by 3D printing, which had good bioactivity and biocompatibility, and the release period of growth factor (BMP2 or P24) was lasted about 30 days. In addition, a new type of bone repair material was composited by beta-TCP porous scaffold material and PLGA microspheres loaded with rhBMP-2. The PLGA microspheres were uniformly distributed in the beta-TCP porous scaffold. The composite bone material particle size was 410 - 820 μm, the porosity was 62% - 75%, and the average compressive strength was 2.18MPa. It had excellent osteoinductivity.

In the research and development process of two kinds of bone repair products, two related Chinese patents were applied. And the patent numbers were 201410301553.0 and 201010106131.X, respectively. Bone repair products have been researched completely, however, the clinical program needs an extended period of time to be approved by the CFDA because that it is a high-risk medical products. China is a populous country and occupies the second largest medical market over the world, so the products have a great market prospect.

Description of the potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main

dissemination activities and the exploitation of results

This challenging project has bundled existing excellence in Europe and China. It is expected that the materials and technologies developed in this project will have a great socio-economic and wider societal impact, as follows.

SOCIO-ECONOMIC IMPACT:

The socio-economic impact of this project can be summarized as follows:

1. The developed techniques for the production of novel (bio)printable materials (bio-polymers and advanced ceramics, as well as hybrid materials) bring high added value products in the health sector.

2. The results of this project enables the fabrication of patient-customized scaffolds/implants that are – for the first time – morphogenetically active for application in orthopedics (bone tissue engineering) and dentistry in a global market.

3. The transfer of the advanced technologies developed in this project into the medical product market increases the worldwide competitiveness of European SMEs.

4. This project strengthen the EU's competitiveness and leadership in science and technology within the international community.

WIDER SOCIETAL IMPLICATIONS:

The wider societal implications of this project are the result of:

1. Development of smart biomaterials which are able to induce the growth and differentiation of cells in a spatially and temporally coordinated way, without the need of addition of exogenous growth factors/cytokines.

2. Fabrication of customizable implants for individual patients under physiological conditions, avoiding complications after surgery caused by nonadequate implant morphology and function, reducing in the duration of treatments and increasing the patient recovery rate.

3. Improvement of the quality of life of patients who suffer from bone defects and disorders, such as traumatic or osteoporotic bone fractures which are increasing in the aging societies worldwide.

MAIN DISSEMINATION ACTIVITIES:

The Dissemination and Communication activities of of Bio-Scaffolds project included publications in refereed scientific journals (at the end of the project, already 33 publications in high-impact journals), presentations at scientific meetings (in total 39 presentations), the publication of a book on "Biomedical Inorganic Polymers", presentations at business congresses and industrial fairs (Medtech Rhineland-Palatinate and CHINA HI-TECH FAIR in Shenzhen, China, a dissemination activity on "3D-Printing and Application in Tissue Engineering and Repair" during the Spring

Meeting of the ZKI-AK, demonstration and dissemination activities in the frame of the joint Bio-Scaffolds Workshops and Summer schools of the European consortium and the Chinese consortium in Beijing, Gothenburg, Brno and Badajoz, the establishment and maintainance of the project website, the distribution of leaflets, brochures, and CD-Roms, various contributions to newspapers, journals and radio (e.g., an article about Bio-Scaffolds in the EU Horizon Magazine), as well as the organisation of the 2014 International CEITEC Annual Conference on “Frontiers in Life and Materials Sciences” in Brno, Czech Republic, and the 2015 International CEITEC Conference “Creating Life in 3D” in Brno, Czech Republic, and of the 1st International Conference on 3D Printing in Medicine in 2016 in Mainz, Germany, presentations to the general public (several activities in radio, newspapers, exhibitions; e.g., during MedTech Rhineland-Palatinate), an exhibition on 3D printing in Mainz Museum and, together with the BioMATiCS group at Mainz University/University Medical Center organization of a public Night Lecture about “From Molecule Biology to 3D Printing”demonstration and dissemination activities in the frame of the joint Bio-Scaffolds Workshop of the European consortium and the Chinese consortium in Beijing, the maintainance of the project website, the distribution of leaflets, brochures, and CD-Roms.

In detail:

- **Workshop I in Gothenburg, Sweden**

This Workshop was held in Gothenburg (ORTOMA) on 2014 23-25 April 2014. The aim of this workshop was to introduce the audience, consisting both of guests/scientists mainly from University and of the project participants, in the state-of-the-art of rapid prototyping technologies and their potential applications in biomedicine/orthopaedics. The second aim of this workshop was to disseminate the results of the project at the host institution. The current problems in the application of rapid prototyping technologies and the contribution of the Bio-Scaffolds in bringing a solution of these problems were discussed. The technologies explained and discussed with the workshop participants included 3D printing and 3D cell printing (bioprinting) (**UMC-Mainz** and **NANOTEC**), robocasting (**UEX**) and the Selective Laser Melting (**THU-1**). The importance of the materials used were highlighted (**UMC-Mainz**, **BUT**, **UEX**, **THU-1** and **-2**, **PKU**, **NANOTEC**, **ORTOMA**, **LANDO**).

- **Workshop II in Beijing, China**

This Workshop was held in Beijing, China (**THU**) on 2015 16-18 April 2015. The aim of this workshop was to disseminate the results and achievements of the Bio-Scaffolds project in China. In lectures and discussion rounds, the products of Bio-Scaffolds project were highlighted. The feedback from the Chinese experts was helpful for the European partners to improve the products of the project. This workshop will surely make it easier to bring, in future, our products to the Chinese market, the largest market in the world, but also help to enter into the market in Europe and other countries. During this workshop, three sessions on Imaging, 3D Printing and Scaffolds evaluation/testing have been organized, including site visits to the respective institutions of the Chinese partners.

- **Summer school I in Brno, Czech Republic**

The Bio-Scaffolds Summer School I was hold in Brno, Czech Republic (BUT) from 2-6 September 2015. This Summer school was also be open for guests from universities and industry from outside the project. Presentations to the broader public included the topics: Biomaterials and 3D-printing (**UMC-Mainz**), Biosilica-loaded nanofiber mats as

morphogenetically active surface scaffold (**UMC-Mainz** and **NANOTEC**), 3D Modeling and visualization of patient-specific brain structure based on MR images (**THU-2**), Bioactive and biodegradable silica biomaterial for bone regeneration (**UMC-Mainz**), Biocalcite as a morphogenetic biomaterial in bone regeneration (**UMC-Mainz**), PLLA/nacre and pearl powder scaffold for bone repair (**THU-1**), Accuracy of digital impressions and fitness of single crowns based on digital impressions (**PKU**), Role of pores in preparation of transparent zirconia nanoceramics (**BUT**), Robocast 45S5 scaffolds coated with HA-PCL composites (**UEX**), Shaping of bioceramics via dip-coating process (**BUT**), Calcium phosphate scaffolds with enhanced mechanical properties (**BUT**), Calcium stabilized ZrO2 ceramics nanocrystals for bioaplications (**BUT**), and Modulation of roughness of Ca-phosphate biomaterials (**BUT**).

- **Summer school II and Final Symposium in Badajoz, Spain**

The Bio-Scaffolds Summer School II was hold in Badajoz (**UEX**) from 23-25 May 2016. This Summer school was also be open for guests from universities and industry from outside the project. Presentations given to the broad public of this free-attendance student-oriented event included the topics: Graphene oxide and reduced graphene oxide used in scaffolds for regenerative medicine (Abalonyx AS, Norway; guest), Morphogenetically active biomaterials (**UMC-Mainz**), E-learning serious game for surgical skills training (Center for Minimally Invasive Surgery, CCMI, Spain), Silicatein, the first enzyme catalyzing a bioinorganic material formation (**NANOTEC)**, Effects of nanoparticles on human mesenchymal stem cells (**THU-1**), Building of 3D microchannel structure for nutrients delivery (**UEX**), 3D modeling and visualization of brain structure and vessels for surgical planning (**THU-2**), CFDA regulations on 3D printing products (**LANDO/THU-1**), and Effect of coating on the mechanical behavior and bioactivity of robocast bioceramic scaffolds (**UEX**).

- **Dissemination activity in the frame of the Spring Meeting of the ZKI-AK (Centers for Communication and Information Processing) - Multimedia & Graphics**

H.C. Schröder (**NANOTEC**) gave a lecture about „3D-Printing and Application in Tissue Engineering and Repair" to a broader audience (non-experts) in the frame of the Spring Meeting of the ZKI-AK (Centers for Communication and Information Processing) - Multimedia & Graphics in Mainz, 11/06/2014 – 13/06/2014.

Subsequent to this meeting, a demonstration of the 3D printers (ZCorporation) and 3D cell printers (bioprinting machine from EnvisionTEC) of **NANOTEC** and **UMC-Mainz** has been organized at the Institute for Physiological Chemistry of the University Medical Center – Johannes Gutenberg University Mainz, organized by **NANOTEC**. More than 30 participants from various universities and companies.

- **CEITEC Conference "Frontiers in Material and Life sciences: Creating Life in 3D"**

BUT was organizer of the CEITEC Conference "Frontiers in Material and Life sciences: Creating Life in 3D", 2 to 4 September 2015, in Brno, Czech Republic. (http://www.ceitec.eu/creating-life-in-3d). The aim of this conference was to explore the interface of the life sciences and material sciences. The program included both plenary sessions and poster sessions. Presentation have been given by several members of the Bio-Scaffolds consortium, both from Europe and from China.

- **1st International Conference on 3D Printing in Medicine**

W.E.G. Müller and X.H. Wang (**UMC-Mainz**) were co-organizers and chairmen of the **1st International Conference on 3D Printing in Medicine** in Mainz, Germany, April 15-16, 2016. W.E.G. Müller (**UMC-Mainz**) gave a lecture about "Advanced bio-ink and 3D-(bio)-printing: New concept for regenerative medicine" and X.H Wang (**UMC-Mainz**) gave lecture about "Morphogenetically active biomaterials: An end to end solution for bone regeneration".

- **Deutsche Biotechnologietage 2014**

W.E.G. Müller and X.H. Wang (**UMC-Mainz**) had the possibility to disseminate the results of Bio-Scaffolds project during the Deutsche Biotechnologietage 2014, April 9-10, 2014, Hamburg, Germany. W.E.G. Müller gave a presentation about "Morphogenetisch aktives Bio-Scaffold für das Tissue Engineering von Knochengewebe".

- **European Chapter Meeting of the Tissue Engineering & Regenerative Medicine International Society**

In addition, W.E.G. Müller and X.H. Wang (**UMC-Mainz**) had the opportunity to present the Bio-Scaffolds project during the European Chapter Meeting of the Tissue Engineering & Regenerative Medicine International Society in Genova, Italy, held from 10-13 June, 2014. The Keynote presentation was given by W.E.G. Müller about the topic: "Morphogenetically Active Bio-Scaffolds for Bone Tissue Engineering". In addition, Xiaohong Wang gave an oral presentation about "Biocalcite, a multifunctional inorganic polymer: Bioseed for the synthesis of calcium phosphate-based bone".

- **BIOMAT 2015**

A further dissemination activity by W.E.G. Müller and X.H. Wang (**UMC-Mainz**) has ben held in the BIOMAT 2015, October 12-14, 2015, Ile de Ré, France. Werner E. G. Müller had an oral presentation about "Biomaterials: Innovative applications in regenerative medicine".

- **WMF Forum 2016**

W.E.G. Müller and X.H. Wang participated and were speakers in the WMF Forum 2016 ("From Global Challenges to Grand Manufacturing Opportunities: Leading towards Growth and Sustainability"), Barcelona, Spain, 3-4 May 2016.

- **Publication of a book within a progress series:**

A book about "Biomedical Inorganic Polymers" which are used for 3D printing of morphogenetically active scaffolds in Bio-scaffolds projects, has been published: W.E.G. Müller, X.H. Wang and H.C. Schröder (eds.) Biomedical Inorganic Polymers: Bioactivity and Applications of Natural and Synthetic Polymeric Inorganic Molecules. Springer-Press, Berlin, pp. 1-303 (2013).

- **German-Chinese Joint Center for Bio-inspired Materials:**

There are close links between the Bio-Scaffolds project and the German-Chinese Joint Center for Bio-inspired Materials ("Biogeomaterials and Marine Sources for Innovative Applications in Biomedicine and Biogeosciences") which is also cordordinated by the Bio-Scaffold's coordinator W.E.G. Müller and X.H. Wang (scientific coordinator) at **UMC-Mainz**.

Presentations at business congresses and industrial fairs

The partners actively participated in the following business congresses and industrial fairs:

- **Medtech Rhineland-Palatinate 2014**

UMC-Mainz and **NANOTEC** presented the results of Bio-Scaffold to the public in the frame of the exhibition Medtech Rhineland-Palatinate with the Ministry of Economy of the state, Eveline Lemke, in Mainz, Academy of Science and Literature, on 18 June 2014. The different steps for the fabrication of customized 3D scaffolds were demonstrated to a broader audience.

- **Participation in CHINA HI-TECH FAIR, November 16-21, 2014, in Shenzhen, China**

The SME partner **NANOTEC**, in cooperation with **UMC-Mainz**, participated in the CHINA HI-TECH FAIR, November 16-21, 2014, in Shenzhen, China, Convention & Exhibition Center.

- **Medtech Rhineland-Palatinate 2015**

UMC-Mainz and **NANOTEC** also presented the results of Bio-Scaffolds to the public in the frame of the exhibition Medtech Rhineland-Palatinate 2015 in Mainz, Academy of Science and Literature, on 8 July 2015. Both institutions are also winners in the national competition "Germany – Land of Ideas". The advantages of the novel morphogenetically active implant materials and the procedure for the fabrication of customized 3D scaffolds were demonstrated.

Distribution of leaflets, brochures, and CD-Roms

Distribution of a number of leaflets, brochures, and CD-roms about the research topic of the project Bio-Scaffolds, for example in the frame of the exhibition Medtech Rhineland-Palatinate, or to guest during the workshops and the summer school.

Contributions to press, TV, broadcast

Several contributions to press and broadcast; for example, project goals and activities were disseminated in various newspapers and journals as well as in radio interviews.

Examples:

Horizon Magazine: An article of the coordinator (W.E.G. Müller) about the Bio-

Scaffolds project ("3D-printed bones could be used to reconstruct faces") has been published in the Horizon Magazine of the European Commission on 07/04/2014.

Night Lecture

Together with his colleagues from BioMATiCS, W.E.G. Müller (**UMC-Mainz**) gave a public lecture about "From Molecule Biology to 3D Printing" in Mainz, Germany, May 12, 2016.

Training activities

Training activies have been performed in association with the project plenary project meetings and Workshops/Summer schools in Beijing, Gothenburg, Brno and Badajoz. During these events, all partners from Europe and China gave presentation according to their expertise and the work plan within this project. In addition, co-workers of the partners had the possibility to exchange knowledge and widen their horizon. The SMEs partners used these meetings to share their expertise with IPR issues and bringing the products into the market.

- **Seminar series for Master students: Invitation of W.E.G. Müller (UMC-Mainz) as a guest speaker at the Justus Liebig University Gießen**

W.E.G. Müller (**UMC-Mainz**) has been invited to present a seminar at the Justus Liebig University Gießen (Germany) on 11.04.2016 in the frame of a Seminar series for Master students in Biology (invitation by the students). The topic was: "Nature's blueprint: Biomaterials for Innovative and Regenerative Implants" Audience: Mainly Master students in Biologie, ca. 100 persons.

- **Highlights (Dissemination and Communication activities)**:

  - More than 30 publications in high-impact journals such as Trends Biotechnol. (IF 10.655) (**UMC-Mainz**), Biomaterials (IF 8.312) (**UMC-Mainz**), J. Mater. Chem. B (IF 6.626) (**UMC-Mainz**) and Bone (IF 4.461) (**UMC-Mainz**)

  - Book on "Biomedical Inorganic Polymers" used in Bio-Scaffolds (eds: W.E.G. Müller, X.H. Wang and H.C. Schröder) in Springer-Press, Berlin

  - About 40 presentations at international and national conferences, and workshops (18 presentations during the last 18-months period)

  - Organizing two international conferences by BUT (CEITEC Annual Conference "Frontiers in Material and Life sciences", 21-24 October 2014, and CEITEC Conference "Creating Life in 3D", 2-4 September 2015, in Brno, Czech Republic)

  - Organizing international conference by **UMC-Mainz** (1st International Conference on 3D Printing in Medicine, Mainz, Germany, April 15-16, 2016)

  - Participtation in international fairs: **NANOTEC** has been selected by the German Ministry for Reasearch (BMBF) as a German representative at the CHINA HI-TECH FAIR, November 16-21, 2014, in Shenzhen, China

  - Together with the **UMC-Mainz** Research Group BioMATiCS, organization of a public Night Lecture about "From Molecule Biology to 3D Printing" in Mainz,

Germany, May 12, 2016

- New patent applications (PCT applications) related to the results of Bio-Scaffolds project have been filed or are in preparation

- Presentations to the general public (several activities in radio, newspapers, exhibitions; e.g., during MedTech Rhineland-Palatinate and in the Horizon Magazine of the European Commission)

EXPLOITATION OF RESULTS:

In order to achieve the application-oriented objectives of this project, besides of the outstanding academic partner institutions, two research-based SMEs at the European side and one successful company in the field of tissue engineering at the Chinese side had been included. So it was feasible to bring our breakthrough discoveries into innovative products, and will be further brought to the market and clinics. The expertise of the European SME partners in the fields of development of pre-operative planning software for 3D modelling (ORTOMA) and in the development of morphogenetically active inorganic polymers for novel types of scaffold materials (**NANOTEC**) facilitated a target-oriented dissemination and exploitation strategy for the new materials and technologies developed in this project.

This project included both exploitable R&D results and contributions to the general advancement of knowledge.

The main commercially exploitable foreground achieved in this project can be summarized as follows:

- The first morphogenetically active cell-containing hydrogel for 3D bioprinting of bone tissue (**UMC-Mainz** and **NANOTEC**)

This exploitable foreground consists of 3D bioprinting bone-forming cells encapsulated into an alginate or alginate/gelatin hydrogel and the subsequent deposition of a morphogenetically active polymer, calcium-polyP or biosilica onto the bioprinted blocks. The bone cells encapsulated into the bioprinted scaffold are functionally active and able to proliferate and form new bone. For further development to apply this method in bone tissue engineering and repair, further investments are needed. We are looking for for VC money or cooperation with a larger company.

- A 3D bio-printed bioglass-containing scaffold consisting of a cell-containing, morphogenically active, polyP- or biosilica-containing alginate/gelatin hydrogel (**UMC-Mainz** and **NANOTEC**)

The developed system consists of a bioprintable and biodegradable cell-containing alginate or alginate/gelatin hydrogel, surrounded by a printable bioglass-containing matrix. The morphogenic activity of the calcium-polyP complex or biosilica is enhanced in a synergistic manner by the integrated bioglass component. Additional investment is needed.

- Morphogenetically active material based on nano/microparticulate Ca-polyP (**UMC-Mainz** and **NANOTEC**)

This novel material for fabrication of customized scaffolds/implants in bone tissue engineering and repair contains morphogenetically active Ca-polyphosphate nano/microparticles. Possible applications are in the field of orthopedics and dentistry, e.g. for sealing of teeth. The commercial use of the material is planned in 2018 and depends on further investments (VC or from other industrial partners).

- Bioprintable, biodegradable and morphogenetically active hydrogel scaffold based on polyP/CM-chitosan/alginate (**UMC-Mainz** and **NANOTEC**)

This novel material composed of a modified chitosan, alginate and polyP can be hardened in a controlled way. Animal experiments revealed that implants containing this material show a much faster healing period of bone defects compared to control implants containing beta-tricalcium phosphate (beta-TCP). Further investments are required (VC or from other industrial partners). The intended commercial use is in 2018.

- Material/beads containing encapsulated silicatein and silica for treatment of bone defects (**UMC-Mainz** and **NANOTEC**)

This material developed by **UMC-Mainz** and **NANOTEC** consists of a combination of silica and silicatein. Both components are encapculated into beads formed by PLGA. Animal experiments demonstrated that also this meterial is superior to beta-tricalcium phosphate (beta-TCP). We are looking for potential investors. The commercial use may also be possible in 2018.

- Microscopy analysis unit (**BUT**)

The developed microscopy image analysis system allows the control of the dip-coating process of very thin fibers. This device can already be produced on demand with a competitive price. A demonstration video is available.

- Custom-made hydroxyapatite bioscaffold for bone regeneration (**BUT**)

A novel manufacturing method based on subtractive manufacturing of customized hydroxyapatite bioscaffolds for bone regeneration is offered by **BUT** and planned to be commercialized in 2017.

- Ultrathin porous and hollow fibers (**BUT**)

BUT will continue to explore the potential applications in soft and hard tissue engineering of its developed fabrication procedure of ultra-thin porous and hollow ceramic fibers. The commercialization of this novel technique is planned in 5 years.

- Dip-coating device for coating of very thin polymeric fibers (**BUT**)

BUT has developed a system for dip-coating of very thin polymeric fibers. A prototype of the device has already been produced and is available for demonstration. This dip-coating unit is already ready to be produced on demand.

- Materials with polymeric or polymer/ceramic composite coatings for bone tissue engineering in load-bearing regions (**UEX**)

The developed impregnation of ceramic/polymer composite scaffolds with biodegradable polymers allows the fabrication of materials with increased strength and toughness. These materials might be suitable for the manufacture of temporary fixation devices, including screws and plates, for future orthopedic applications. Negotiations with partners potentially interested in the technology are underway.

- Robocast scaffolds from calcium phosphate (hydroxyapatite, HAp, and TCP) nanopowders (**UEX**)

These materials are of interest as scaffolds for bone tissue engineering and as bone-graft substitutes. The commercialization in the fields of orthopedics/dentistry might be possible after 3-4 years after finding potential investors or industrial partners.

- Ink composition and fabrication protocols for 3D printing of complex structures by robocasting (**UEX**)

The commercial use of the optimized robocasting ink compositions (reaching from the use of graphite to polymers) for additive manufacturing of complex structures is planned in 2-3 years. First negotiations with potentially interested partners from industry have been initiated.

- Imaging system (**ORTOMA**)

The imaging system developed by **ORTOMA** allows the collection of medical data from CT/MRI and extraction of geometry data with high resolution for CAD/CAM based processes. This imaging system (3 patent applications) has alredy been successfully tested in a hospital. It has also undergone CE marking. The developed interface to export data to manufacturing system and to navigation system fulfils the demands from FDA and medical device directive. At present, **ORTOMA** is negotiating with several hospitals to introduce the system into the market. The commercial use is planned in already 2017.

There is also a number of exploitable foreground that has brought a significant progress in general advancement of knowledge, but is not foreseen for direct commercial application. These techniques comprise, for example, a new fabrication (sintering) procedure for the production of 45S5 Bioglass structures at lower temperature (**UEX**), a method for coating of scaffolds (hydroxyapatite, bioglass, and titanium) with a thin layer of calcium-polyP (**BUT**), and a technique for the fabrication (via coaxial deposition) of robocast scaffolds with a continuous internal channel for potential application as nerve guides or for new bioreactors (**UEX**). The patentability and future applications are analyzed.

THE MAIN ACHIEVEMENT AND BREAKTHROUGH of Bio-Scaffolds project is surely the fact that the consortium successfully developed, FOR THE FIRST TIME, (i) NOVEL SCAFFOLD MATERIALS THAT ARE MORPHOGENETICALLY ACTIVE and can be integrated into the RAPID PROTOTYPING CHAIN for the FABRICATION OF SCAFFOLDS/IMPLANTS THAT DO NOT NEED THE ADDITION OF GROWTH FACTORS AND CYTOKINES, and (ii) PRINTABLE CELL-CONTAINING 3D

SCAFFOLDs THAT ALLOW THE CELLS (BONE FORMING CELLS) TO GROW AND TO DIFFERENTIATE.

Future developments will especially focus on:

1. 3D printing of orthopedic and dental implants
2. Fabrication of novel regeneratively active biomaterials based on natural biopolymers
3. Looking for venture capital for development of bone and dental implants

Website address:

http://www.bioscaffolds.eu/

Contact details:

Partner 1 (UMC-Mainz) - Coordinator:
Prof. Dr. Werner E. G. Müller [UMC-Mainz-1]
ERC Advanced Investigator Group
Universitätsmedizin der Johannes Gutenberg Universität
Institut für Physiologische Chemie
Duesbergweg 6
55128 Mainz, Germany
Phone: +49-6131-3925910
E-mail: wmueller @uni-mainz.de
Germane-Chinese Joint Lab "BioNanoComposites":
Prof. Dr. Xiaohong Wang [UMC-Mainz-2]
ERC Advanced Investigator Group (W.E.G. Müller)
Universitätsmedizin der Johannes Gutenberg Universität
Institut für Physiologische Chemie
Duesbergweg 6
55128 Mainz, Germany
Phone: +49-6131-3925789
E-mail: wxh0408@hotmail.com

Partner 2 (BUT):
Prof. Dr. Karel Maca
Prof. Dr. Martin Trunec
Dr. David Salamon
Department of Ceramics and Polymers
Institute of Materials Science and Engineering
Brno University of Technology
Technicka 2
61669 Brno
Czech Republic
Phone: +420 541-14-3344
e-mail: maca@fme.vutbr.cz

e-mail: trunec@fme.vutbr.cz
e-mail: david.salamon@ceitec.vutbr.cz

Partner 3 (UEX):
Prof. Dr. Pedro Miranda González
Departamento de Ingeniería Mecánica, Energética y de los Materiales
Escuela de Ingenierías Industriales
Universidad de Extremadura
Avda. de Elvas
06006 Badajoz
Spain
Phone: +34 924 28 96 00 (Ext. 86735)
E-mail: pmiranda@unex.es

Partner 4 (ORTOMA):
Matts Andersson, CEO
Ortoma AB
Erik Dahlbergsgatan 11A
41126 Göteborg
Sweden
Phone: 46-3-17-735163
e-mail: Matts.andersson@ortoma.com

Partner 5 (NANOTEC):
Prof. Dr. Dr. Heinz C. Schröder
NanotecMARIN GmbH
Duesbergweg 6
D-55128 Mainz
Germany
Tel.: +49 6131-39-25791
e-mail: hschroed@uni-mainz.de

Partner 6 (THU-1):
Prof. Dr. Zhijian Shen
Prof. Dr. Qingling Feng
Department of Materials Science & Engineering
Tsinghua University
Beijing 100084
P.R. China
Phone: +86-10-62782770
e-mail: biomater@mail.tsinghua.edu.cn

Partner 6 (THU-2):
Prof. Dr. Guangzhi Wang
Department of Biomedical Engineering
Center for Biomedical Imaging Research, Tsinghua University
Tsinghua University School of Medicine
Beijing
P.R. China
e-mail: wgz-dea@tsinghua.edu.cn

Partner 7 (PKU):
Prof. Dr. Yihong Liu
School and Hospital of Stomatology
National Engineering Laboratory for Digital and Material Technology of Stomatology
Peking University

Zhongguancun Nandajie No.22,
Beijing, 100081
P.R. China
e-mail: kqliuyh@163.com

Partner 8 (LANDO):
Dr. Rongwei Tan
Shenzhen Lando Biomaterials Co.,Ltd
High-Tech Industrial Park
Nanshan District
Shenzhen
P.R. China
Phone: +86-755-86368970
E-mail: tanrw@landobiom.com
Dr. Zhending She
Shenzhen Lando Biomaterials Co.,Ltd
High-Tech Industrial Park
Nanshan District
Shenzhen
P.R. China
Phone: +86-755-86368970
E-mail: shezd@landobiom.com