Final publishable summary report

Executive summary

SMILEY project intended to apply a bio-inspired assembling/mineralization process (MIAO) to a wide number of macromolecular matrices with the purpose to generate new smart devices with application in several high-impact fields. The process was previously well assessed on collagen molecules: under physiological conditions and by pH variation, collagen fibrils assemble into thicker fibres and undergo mineralization with biomimetic apatite nanophases, thus generating fibrous scaffolds for bone and osteochondral regeneration with outstanding mimicry of the host tissues, and high regenerative ability. The MIAO process is entirely driven by the information and control mechanisms inherent in the molecular structure of collagen and by the features of biomimetic apatites that nucleate in specific molecular loci present in the collagen structure, thus reproducing the biological phenomena of the formation and mineralization of hard connective tissues in mammals. SMILEY intended to translate this process towards wider applications, by exploiting the ability of natural polymers and fibres to expose functional groups enhancing chemical, or physical, mutual interaction. This opens to the generation of macromolecular composite matrices where bio-inspired mineralization may occur. In this respect, apatites are a family of minerals exhibiting a variety of properties, as induced by the ions present in its lattice. Therefore SMILEY aimed to direct and tailor MIAO processes to the generation of hybrid composites with designed properties for multiple, high impact applications in the environmental, health and safety fields. Particularly, SMILEY aimed to response to relevant socio-economic needs such as: i) the filtration of nanoparticles in critical size range, ii) the regeneration of dental tissues such as periodontium and dentine and, iii) the production of energy by wearable, low cost devices. SMILEY focused its activity in the selection and modification of natural polymers and fibres to create macromolecular matrices with designed properties. The association of polymers with different hydrophilic ability enabled the synthesis of 3D constructs with ability to recover and manage the moisture, for application in healthcare as Heat Moisture Exchanger devices (HME). Bio-inspired mineralization with apatite phases was carried out in compliance with the requirements for the final devices. Therefore, biomimetic scaffolds mimicking the whole periodontium and the dentine were developed and biologically tested, thus opening to new possible solutions for regeneration of the whole tooth. A new apatite phase with photoelectronic properties was developed and firstly nucleated onto natural fibres to obtain photo-active fibrous elements that were incorporated into flexible devices able to produce energy. Iron-substituted hydroxyapatite nanophase was developed and tested in all the three application lines to exploit its properties, by means of hyperthermia, possibly activating thermophoretic effects in filters, cell stimulation towards enhanced bone formation, and possible electronic properties. The MIAO processes yielding the three different devices were analysed to draw preliminary roadmaps for the development of flexible production lines for the manufacturing of smart devices for mass applications in relevant socio-economic fields.

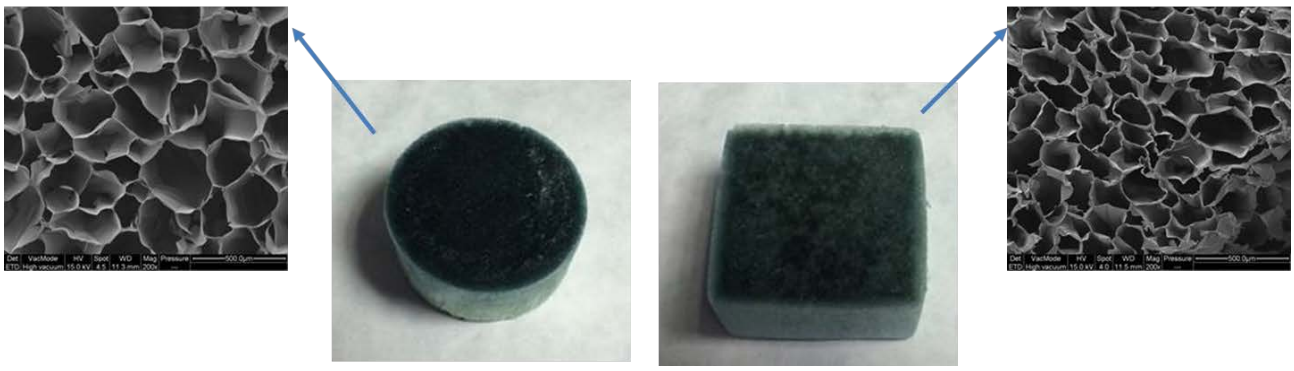
Summary description of project context and objectives

The project SMILEY pursue the development of new materials to solve relevant socio-economic needs, as following described.

•***Smart filtering devices.***

In the last decade the contamination by airborne particles has become a crucial aspect in human health due to recent research and findings that point out potential and/or effective physical damage. The harmfulness of contaminating media is strongly increased in the case of nano-sized particles, where it is related to the particle size, specific surface area and surface activity. It has been shown that cells and organs can give a toxic response even to apparently non-toxic substances, when they are exposed to a sufficient dose in the nanometric size range. Filtration is the simplest and most common method for particle removal from air (or other gases) and it is used in a variety of applications. The filtering mechanism can be simply mechanic (commonly by using glass fibres) or assisted by permanent (electret filters) or activated electric fields (electrically enhanced filter, EEF). The most important characteristics of air filters are the removal efficiency and flow resistance or pressure drop. None of the known mechanisms for particle interception is dominant in the intermediate region (typically ranging between 30 and 500 nm), commonly called the most penetrating particle size (MPPS). On the basis of recent results, efficient filtering devices for personal protection against nanopowders generated in battlefields can be obtained by MIAO processes applied to gelatine-based matrices bio-mineralized with apatite nano-particles. This preliminary result represents a conceptual pin for further development of innovative hybrid filtering devices whose performance can be tailored and directed by flexible adjustment of the synthesis parameters.

Porous structures functioning as fibrous filtering devices

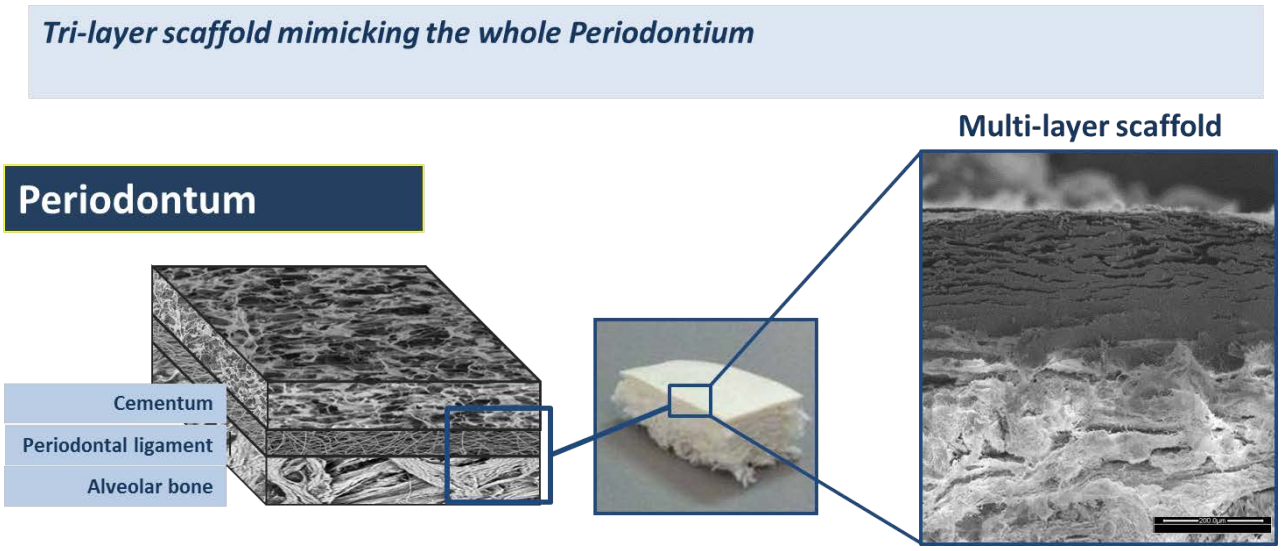


HME filters are used in artificial ventilation to humidify and warm the cold and dry gases from the pulmonary ventilator. Such filters work as heat exchangers, which accumulate the heat delivered from the patient breath and transfer it in the inhalation phase. These filters accumulate the moisture that arise from the breath and then condensate because of the reduction of the external temperature and afterwards the moisture will be transferred during inhalation. These filters work at room temperature and can humidify and heat the inlet gas of a few degrees. Generally HME filters are used in anaesthesia and for short treatments, while in

intensive therapy (long treatment) the use of steam heated water are preferred, because they are better in terms of performance. However they are more complicated (large dead space variables), more subjects to bacteriological pollution and have higher costs. In Europe alone the use of HME filters is estimated as 30 million pieces. The possibility of heating the filter with electromagnetic fields would allow a greater functionality of the filter in terms of heat and hygrometric exchange and with an instant efficiency (the latency of a not heated filter is equal to about one hour). A further function of HME filters, in addition to the properties described above, concerns the antibacterial and antiviral filtering capabilities.

• ***Scaffolds for regeneration of dental tissues***

Like bone, tooth regeneration is of particular relevance to the field of regenerative medicine and elicits a huge public interest. Tooth loss is a common result of a variety of oral diseases due to physiological causes, including dental caries, periodontal disease, trauma, genetic disorders and aging, and can lead to physical and mental suffering that markedly lower the individual's quality of life. A tooth is a complex organ that is composed of calcified tissues (enamel, dentin and cementum), and soft connective tissues (the dental pulp and the periodontal ligaments) in which blood vessels and nerves are protected. Therefore, regenerating a whole tooth for clinical tooth replacement is considered to be a remarkable scientific objective and an outstanding goal. Human teeth form following a complex cascade of biological events that involve several cell lines. Therefore, dental scaffolds should exhibit high compositional and structural mimicry of the different dental tissues.



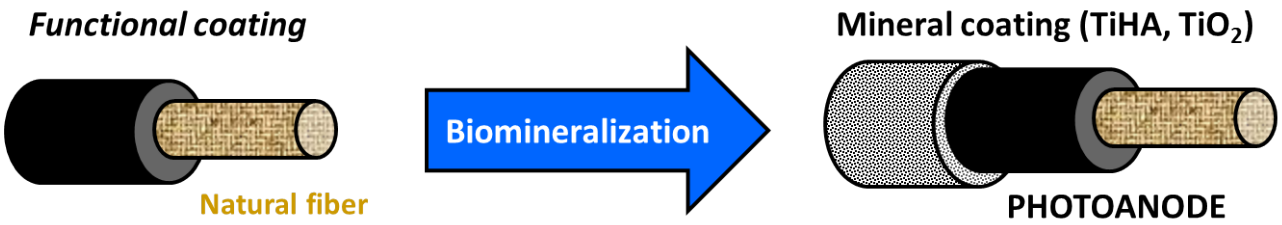
To date, approaches to tooth regeneration are still at their infancy and face many challenges. Current processes are mainly based on biomimetic calcium phosphate coatings used to modify dental titanium implants. More complex approaches have been used to engineer structure containing layers which are targeted at the regeneration of different dental tissues, or even tooth root engineering using tissue from postnatal teeth. However, these approaches still have not provided ECM-like scaffolds which can drive cell behaviour towards tissue regeneration. In this respect, the understanding of the bio-mineralization principles governing tooth formation represents a pivotal step in designing regenerative biomaterials. It is becoming increasingly apparent that the MIAO processes at the basis of the formation of periodontal bone, cementum

and dentin are quite similar to those occurring in bone synthesis. In this respect, in 2001 the proposers (CNR-ISTEC) developed a laboratory procedure which closely resembles the MIAO process naturally occurring in the formation of new bone tissue and which led to highly regenerative bone scaffolds. Upon controlled variation of the mineralization degree, CNR-ISTEC also obtained morphologically and compositionally graded scaffold that, due to its remarkable mimesis with the biological tissues of the articular region (e.g.: subchondral bone, mineralized and hyaline cartilage) was able to trigger the specific cell phenotype of each different tissue and completely regenerate osteochondral defects. The success of these devices led to a number of international patents and commercialized biomedical devices. More recently, the proposers (CNR-ISTEC) developed and patented a process for the synthesis of HA nanopowders with intrinsic super-paramagnetic properties thanks to a chemically and positionally controlled doping of HA lattice with Fe2+/Fe3+ ions. These are promising for the development of biomimetic scaffolds with increased regenerative potential. The recognized success of biomimetic medical devices obtained by MIAO processes suggests that a viable and promising approach for tooth regeneration can be the successful fabrication of ECM-like structures which mimic different tooth areas, together with adequate tissue engineering strategies.

- ***Fibrous photovoltaics for energy production***

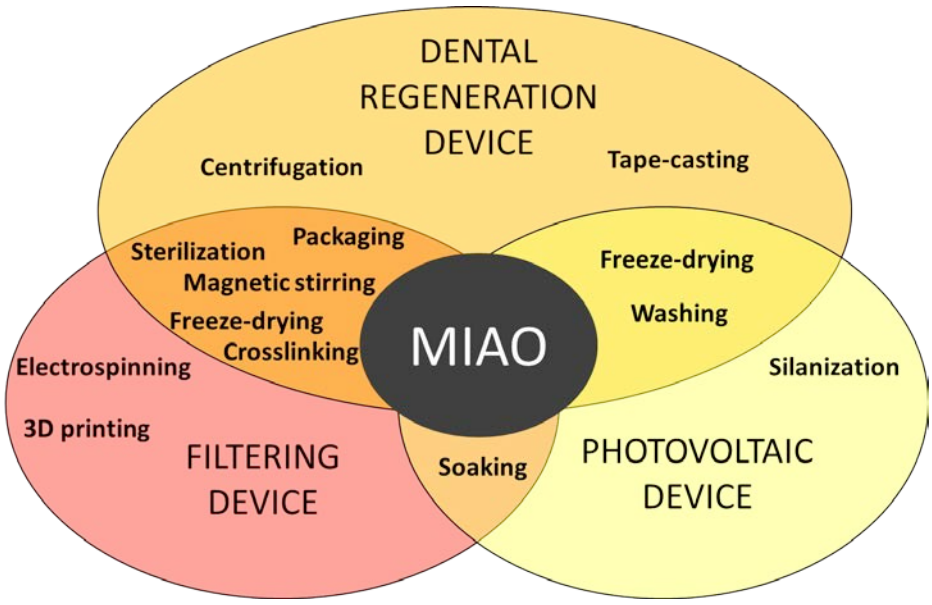
With the increasing concern about environmental issues and growing energy demands, solar energy is considered the most attractive, long term energy source and solar cells the major candidate for its harnessing. Photovoltaics are in fact the most advanced way of providing electricity in the absence of a main supply. The widely used silicon based (mono or polycrystalline) cells are rigid, and are therefore not suitable for curved surfaces and have to be rightly oriented towards the sun in order to assure the desired efficiency. Integration of solar cells into textile would benefit numerous fields including clothing, transportation and civil engineering. Further, with the rise of portable electronic devices, Fabric Integrated Photovoltaic (FIP) can potentially power most mobile electronics (e.g.: mobile phones, tablets, mp3-players). There are limited scientific studies and very few commercial applications of FIP. From the viewpoint of the customer, the FIP should be fully conformable, offer a universal socket for the different devices, and should be well integrated with the specific design of the garments considered. For this reason, research has been focused on developing fibre-based solar cells. When coupled with the commodity scale of fibre and textile technology, fibre-based photovoltaic devices could help to realize cost-effective, scalable solar energy harvesting. Particularly, dye-sensitized solar cells (DSSCs) could offer interesting opportunities in terms of end user satisfaction. The core of the DSSC cell is the semiconductor photo-anode that should exhibit: fast electron injection and separation, slow electron recombination, excellent electron transport, high surface area and outstanding light collection. Besides the conventional TiO2 and ZnO, doped apatites have recently attracted attention for their photo-electrochemical

Properties, also thanks to recent results showing that the band gap of apatites can be easily tuned according to the amount of specific ions introduced into the crystal structure.



Some attempts have been made to produce flexible DSSC fibres, however the obtained substrates did not offer the possibility of integration into garments using the textile technology. MIAO processes are therefore promising as a new approach for synthesis of new flexible photovoltaics for smart textiles.

In respect to the above described issues, SMILEY aimed to develop and apply a "bottom-up" approach to build nano-structured devices with smart, multi-functional properties, by using abundant and environmentally safe raw materials, such as natural polymers and fibres.



Process phases intersection among the three different processes

To this aim, nature was used as the inspiration for the development of an ensemble of MIAO processes, which was controlled to first generate elementary nano-sized building blocks, and then to direct their assembly towards the final devices, in particular:

i) Smart filtering devices

A proper implementation and direction of MIAO processes provided fibrous constructs with tailored pore size, geometry and organization as well as a high degree of mineralization. Macromolecular templates with optimized properties of microstructure, porosity, physical stability in relevant environment and efficiency, were developed and engineered to create fibrous structures with ability of filtration and management of the moisture. Fibrous filters were finally developed as Heat Moisture Exchangers (HME), which can be used for patient external ventilation, to humidify cold and dry gases coming from the pulmonary ventilator.

ii) Scaffolds for dental repair and regeneration

MIAO process in association with other forming techniques, was directed to the development of hybrid mineralized constructs mimicking alveolar bone, periodontal ligament and cementum. The designed constructs, exhibiting physico-chemical-microstructural features mimicking different human mineralized tissues, were also developed in association with superparamagnetic FeHA phase to obtain ability of remote activation by magnetic fields. Hybrid mineralized scaffolds with tubular porosity mimicking dentin tissue were obtained and tested. Stability tests and preliminary biologic analysis confirmed the potential of the new materials to be used as dental scaffolds.

iii) Fibre-Based Photovoltaic Devices

Bio-inspired MIAO processes was carried out in association with various natural fibres, resulting in heterogeneous nucleation of new apatite phases with photovoltaic properties, synthesized and developed in the project. Photoactive components were obtained also by dye staining in mineral crystals. The setup and optimization of the manufacturing process, in compliance with the features of MIAO process, led to development of photovoltaic fabrics replicating, at the fibre level, the structure of third generation solar cells flexible (so-called Dye Sensitized Solar Cells). The potential of integration of photovoltaic devices in fabrics will generate outstanding advantages in all fabric-related applications, for a convenient form of off-grid energy generation.

The success in the direction and application of MIAO-based processes to the development of different devices with designed properties is a first step for the establishment of a technological platform based on highly reproducible, scalable and cost-effective processes for the synthesis of multi-functional devices with huge economic, environmental and social impact.

The development of SMILEY required a multi-disciplinary approach, thus a deep trans-national cooperation and synergy between European Public Research Centres and Universities endowed with experience in Material Science (CNR-ISTEC/ISAC, FSU Jena, IWNiRZ) and basic knowledge on template-mediated mineralization processes (UoY, Univleeds), as well as private companies expert in biomaterials (FINCER) and filtering systems (POLLUTION), and in the assessment of biological characteristics of the obtained devices (LEMI).

Main S&T results/foreground

WP1. Selection and modification of natural polymers/fibers

Objectives

- To provide specifications of the most relevant chemico-physical, morphological and functional properties that 3-D constructs obtained by MIAO process should exhibit in order to function as filtering devices for nanoparticles, scaffolds for dental regeneration or photovoltaic fabrics respectively.
- To select suitable natural polymers and fibres, which will be pre-processed, when necessary, prior to applying MIAO processes. Blends of the obtained raw or pre-processed materials will be achieved to obtain composite polymeric fibrous matrices.

Participants: IWNiRZ, CNR, Univleeds, FSU Jena, FINCER, LEMI, UoY, POLLUTION

Progress of WP1

Blends of natural polymers (i.e. collagen, chitosan, alginate, gelatin, BNC) were studied with the aim to improve chemical and/or mechanical stability of the developed prototype and to dispose of polymeric matrices suitable for MIAO process. Multi-component stable solutions and suspensions were prepared, then the parameters regulating fibration/co-precipitation and assembling of polymers such as temperature, pH, concentration, were setup. Blends stability was optimised in terms, not only of chemical composition, but also of chemical reticulation and drying process. Besides the blends based on natural polymers, less hydrophilic polymers like PCL, PLLA, PLA/PHB resistant to water/humidity, relevant in the case of filtering system, was also investigated. The key goal is to build organized 3D polymeric constructs endowed with the suitable chemical-physical, structural and ultra-structural features inducing the specific functionalities required for each of the tree different application involved in the project. A clearer definition of the relevant raw materials and related modifications for the different final devices was made.

In particular:

Filtering materials

Chitosan and gelatin were selected on the basis of their hydrophobic and hydrophilic properties, suitable to achieve devices for moisture capturing and exchange. Alginate, wool, PLLA, PCL/PHB and BNC were selected as base materials for their potential to create macro- and micro-structured network with organized porosity suitable to capture the nanoparticles.

Dental scaffolds

To achieve suitable mimicking of cement, periodontal ligament and alveolar bone tissues, collagen and bacterial nano-cellulose were selected as base materials. To mimic the complex structure of the dentin alginate and gelatin were choose. These raw materials also offer the possibility of forming complex structures (e.g. tubules, membranes) so to make easier the development of tooth-mimicking constructs.

New DSSCs

Following the activity carried out in the first eighteen months, wool and flax were selected as the most promising natural fibres to drive MIAO processes. Moreover, wool and flax resulted suitable to be coated by a conductive polypyrrole layer that results able to drive biomineralization with mineral phases endowed with the required band-gap value (selected in WP6: titanium doped hydroxyapatite and titanium dioxide).

WP2. Hybrid composites by MIAO process: activation of control mechanism mediating biomineralization

Start month: 1 End Month: 24

Objective

To synthesize hybrid mineralized composites by controlled fibration of different organic polymers / fibres, acting as templates for heterogeneous nucleation of various mineral phases. A continuous feedback between the synthesis conditions and the assessed properties of the hybrid composites will provide a comprehensive picture of the mechanisms controlling assembling, organization and biomineralization processes as well as the chemico-physical, structural and ultra-structural features of the 3-D constructs that determine specific functionality.

Participants: CNR, Univleeds, FSU Jena, IWNiRZ, UoY

Progress of WP2

- Bio-inspired mineralization of several bio-polymeric fibrous composites based on silk, fibroin, cellulose acetate, PLA/PHB copolymers, acetylcellulose and bacterial nanocellulose were investigated, for the heterogeneous nucleation of calcite crystals, by using different habit modifiers to force inorganic crystals to nucleate at fibres surface and to produce adherent, uniform coatings (UnivLeeds). Also, the crystallization of apatite in defined conditions of confinement, also in presence of different agents such as glycine, glutamine and citrate ions, was investigated (UnivLeds, CNR).
- The heterogeneous nucleation of substituted apatites was tested on collagen-based blends and gelatine matrices. In particular, the crystallization of magnesium- and iron-substituted apatite nanoparticles was achieved in different conditions, i.e. different apatite/collagen ratios, to investigate the potential to create fibrous constructs with different mineralization extents for dental reconstruction (CNR).
- Calcium carbonate crystals were modified by incorporation of organic molecules to generate new dyes for use in DSSCs. The use of amino-acids to improve the incorporation of molecule was investigated and some of the mechanisms related to the phenomenon were elucidated.

Cross-linking procedures were investigated and applied to collagen, gelatin, chitosan, alginate and blends of them to impart defined morphologies and chemic-physical properties.

Directional freezing into 2D and 3D patterns was investigated on different polymeric matrices, resulting in the achievement of constructs with defined porosity and promising to create pre-defined 3D ordering into a gel. Heterogeneous nucleation of mineral phases exhibiting photo-electronic properties (e.g. Ti/Zn apatitic-like phases, TiO_2, ZnO) were setup on natural fibers to produce new generation dye-sensitized solar cells.

- The synthesis of new inorganic dyes was investigated by exploring the mechanisms driving incorporation within calcite crystals by mediation of suitable amino acids at definite pH (UnivLeeds). The observed correlation between the pH of the reaction mixture and the level of dye occlusion supported the previously proposed mechanism of dye incorporation which involved the formation of intramolecular interactions between both organic species, and the dependence of the protonation of amino acids by pH. It was also shown that the relative charge of supporting amino acids can be used as a reference value to predict which amino acids are going to enable dyes occlusion at any given conditions. Further, by studies of polarized light it has been shown how some organic dyes orient preferentially along specific crystal directions.

$ZnO\text{-}TiO_2$ composite nanopowders (NPs) were investigated and prepared by laser co-vaporization (CoLAVA) method to develop photoelectric active ceramic semiconductors with minimization of photo-generated electron/hole pairs recombination (FSUJ). These raw powder mixtures were vaporized into plasma in the intense focus of a pulsed CO_2 laser beam in a continuously flowing process gas (air) at atmospheric pressure. The new materials showed increased activity due to intra- or interparticle phase compositions and phase distributions. Both could result in heterojunction effects which increase the lifetime of photo-generated free charge carriers. In consideration of these result, cost efficiency, biological and chemical resistance, a high oxidation potential, and a long-term stability against photo- and chemical corrosion, laser co-vaporized $ZnO\text{-}TiO_2$ composite nanopowders (NPs) are ideal candidates for applications in the environmental sector as well as for immobilization in and photoelectric activation of nano-fibrillar networks or textile fabrics.

The transition of amorphous calcium phosphate (ACP) to HA was studied by TEM (ISTEC-CNR) to study the process of infiltration and mineralization of microfibers such as collagen. The role of the citrate in the stabilization of ACP was studied in details also by Raman microscopy and transmission electron microscopy and electron diffraction on ACP/citrate samples as a function of time. The occurrence of distinct diffraction rings at scattering vectors related to the {002} and {211} lattice planes of HAp was firstly observed, then after approx. 5 days the transition to HAp is complete.

- Fe_2O_3 silica coated LAVA nanoparticles (FSL) with a composition of 60 %v/v Maghemite and 40 %v/v Silica was dispersed in culture media to incorporate in bacterial nanocellulose (BNC) fleeces (FSUJ). Stirring procedures were adopted to significantly enhance the BNC production and improve homogeneity in the BNC network. This should lead to 3-D nanofibrous cellulose hydro-gel hybrid materials with adjustable magnetic properties. Although, the achievable saturation magnetization is directly connected to the stability of the dispersion in the culture medium, and the quantity of incorporated particles, both are on an upper limit of 2 g/L.
- MIAO processes were developed (ISTEC-CNR) to obtain hybrid superparamagnetic composites suitable for moisture exchange and filtering applications (HME devices). Due to its ability to act as a habit modifier in mineralisation processes, by virtue of its strong affinity with divalent ions, alginate was used as a matrix to generate cross-linked 3D structures with resistance to water dissolution but high hydrophilic character. 3D ordered structures composed of alginate xerogels mineralised with FeHA were proven to be suitable internal components for filtration-HME systems, particularly to be used as the core part of HME filters and embedded within hard Gelatine/Chitosan blend, optimized in WP1. Preliminary tests showed a good integration between these two components and confirmed the stability of the magnetic internal part. This suspension was further freeze-dried to obtain a superparamagnetic composite material that could be finally integrated into the final HME filter.
- Mineralization of Type I collagen was optimized to generate a superparamagnetic material with high mimicry of the structure of alveolar bones (ISTEC-CNR). Suitable 3D scaffolds were obtained and optimized by means of composition and physical stability. The tuning of the porosity extent and pore organization ideally favoring cell adhesion and proliferation and scaffold vascularization was obtained by

controlled freeze-drying processes. In order to develop a dentine-mimicking scaffold endowed with magnetic properties, the MIAO process was set up in the presence of gelatin, as a mineralized matrix that was then mixed with an alginate matrix in order to obtain the channel-like morphology typical of dentine.

Mineralization procedures for fibres addressed to Dye-Sensitized Solar Cells (DSSCs) were developed (ISTEC-CNR). Polypyrrole (Ppy) coated fiber composites were produced by in situ chemical oxidative polymerization of pyrrole on the surface of carded wool at room temperature. MIAO process was applied to obtain a layer of hydroxyapatite (HA) and titanium doped hydroxyapatite (Ti-HA), with a composition of 15 mol%, with improved coating uniformity. Pyro-glutamic acid was used to promote HA and Ti-HA nucleation on its surface. A complete covering by the mineral phase was obtained, at high calcium/phosphate concentration and thanks to the use of pyroglutamic acid.

- The systematic changes in the molecular and mechanical properties of the collagen microfibers following mineralization were investigated by Raman microscopy (UoY). It was shown that the proline Raman band shifts as a function of stress only in the presence of mineral phases. Combination of experimental Raman data and the modeling results therefore indicate a significant impact of the degree of mineralization on the molecular configuration of the proline. At this point it might be speculated that the role of mineralization could be to inhibit the possible stress-response of the collagen via the reduced cross-linking potential of the proline, where this would lead to an increased response of the proline to stress.

WP3. Set up of process repeatability and scalability at pilot plant

Start month: 18 End month: 36

Objective

WP3 aims to assess the translatability of the project results toward industrial applications in terms of production and investments, quality, regulatory requirements, and commercial opportunity.

The new fabrication concepts will be transferred to develop flexible production lines producing new custom-made multi-functional products with high level of automation and high product quality.

Participants: FINCER, POLLUTION, CNR, UnivLeeds, FSUJ, IWNiRZ, LEMI

Progress of WP3

The feasibility of modifying the MIAO process in a flexible manner, and of directing the process in a repeatable manner to develop the three different devices envisaged in SMILEY project was thoroughly investigated. A roadmap for development of flexible production lines based on MIAO processes was traced. The MIAO process was flexibly directed to the pilot scale up of the final devices, thus reaching different level of advance. Specific parameters for quality assurance and control in the three production lines were defined, together with relevant Standards of Protocol.

The main steps toward the product commercialization and dissemination were identified, in respect to the features of the different applications. Therefore, some of the reported aspects are related to products designated to the medical field and the related patient health preservation. The process validation and the process scale-up are a common point within the project therefore the intersection among the different processes were defined, to find the common steps. On the other hand, biocompatibility has to be structured taking into account the application and classification of the device, talking about medical devices. The biocompatibility is correlated to the product and ISO 10993 has to be analyzed and followed in order to define the test and path necessary for the application and classification.

Analyzing the whole process and considering the overlapping phases, in the center we can fix the MIAO process related to the biomineralization of natural fibers. In detail, the MIAO process is applied to the dental field with the biomineralization of gelatin for the dentinal scaffold and collagen for the periodontal substitute prototype.

Within the filtering application the wool seems to be the best candidate, also applied for the DSSFs devices which has also tried a proof of concept with flax.

The MIAO process is always characterized by an acid-basic reaction which allows the hydroxyapatite particles to be nucleated on the natural fibers.

Collagen and gelatin need a precipitation of their acidic solution within the basic one, while the wool and flax are dipped and impregnated in the basic solution and then immersed in the acidic one.

What is real in common comparing all the processes within the scale-up and validation phases is the freeze-drying and packaging steps.

MIAO process	Freeze-drying	Packaging
Collagen/Gelatin	✓	✓
Wool	✓	✓
Wool/Flax	✓	

Common steps within the MIAO process

HMEs device

Concerning the industrialization of the natural polymers to be used as HMEs, the critical parameters which need to be monitored in the lab-scale production were accurately defined. Pilot-scale tests were carried out in 2015, to setup and gather optimized protocols to obtain homogeneity and reproducibility of the freeze drying process, as well as the best shape and size for the containers.

Dental scaffolds

Engineering aspects of process scale-up have been analyzed in order to assure a reliable and practical method of manufacture from laboratory into industrial scale facilities. A pilot plant has been designed to produce trial lot quantities of material for preclinical characterization. The pilot plant has been useful to understand critical process parameters and lead to the definition of standard operation procedures for batch production of

material and relative quality controls. Test data demonstrate that product specification have been maintained. TGA, ICP analysis, and swelling test data showed the conformity of the values and the repeatability of the manufacturing process taking into account all the scale-up activities developed within WP3.

Fibrous Photovoltaic Devices

The synthesis of blue, dyed, zinc oxide crystals using the described methodology is the first successful example of organic dye incorporation within inorganic mineral that took place in the microwave radiation field. It is considered to be a breakthrough, as it enables other researchers to produce high quantities of artificially coloured, semiconducting inorganic solids. The main success of the work came from developing a system that allowed for the synthesis of dye-doped zinc oxide with microwave heating, rather than conventional. This was significant as the reactions were completed at times as short as 1 minute, compared to 30 minutes with conventional heating. Then as the temperature and time of the reaction in the microwave increases, so does the number of particles present in the product.

Quality Assurance

Fibrous Filters

Several International Standards were taken into consideration to develop and optimize the two experimental setup to test filtration and humidification exchange efficiencies of SMILEY samples at ISAC-CNR and Pollution Srl, respectively.

Concerning filtration efficiency properties, the certification process and the requirements for quality control testing of respiratory protective devices are regulated by three different organizations: ISO (International Organization for Standardization), CEN (European Committee for Standardization) and NIOSH (American National Institute for Occupational Safety and Health). The Standards which were necessary to consider to define the parameters and the equipment of the experimental tests were defined. The quality factor (QF), defined as the filtration capabilities of each sample against its pressure drop, was measured and the performance of each sample was classified and compared with other commercial filters.

Certification process and requirements for quality control testing of materials to be used as HMEs were based on the gravimetric method suggested by ISO Standard 9360-1 and 9360-2 for Anaesthetic and respiratory equipment – Heat and moisture exchangers (HMEs) for humidifying respired gases in humans. Moreover, Pollution combined this standardized gravimetric method with the hygrometric method. By the end of the project, a new experimental setup combining both methods was developed and optimized.

Another important issue regarding the quality control of these materials was the sterilization process. It was decided to proceed with the γ-rays sterilization. The sterilization process was carried out with a gelatin-chitosan sample, a PLA/PHB with QACs, Cellulose Acetate (CA) membranes and a PLA 3-ways sample holder made with the 3D printer. Visual inspection showed no significant differences in the material structure. In addition, experimental results before and after the sterilization were exactly the same, confirming the good structural stability of these samples against the γ-rays sterilization process.

Dental scaffold

This section is focused on the set of Standard Operating Procedures to assess the quality of all systems (equipment used, staff training, selection of quality materials, manufacturing processes) in the lines for production of MIAO-derived devices. SOPs have been focused on: documentation management, suppliers' qualification and management, personnel training, equipment control, calibration and maintenance, guidelines for the management and writing of qualification and validation documentation.

Fibrous Photovoltaic Devices

A set of SOPs for quality control of fibrous photovoltaic fabrics have been written. In particular the SOPs concern: the deposition of PPy on yarns, the biomineralization processes (TiHA) on conductive cashmere yarns, the biomineralization processes (Ti-F-HA) on conductive cashmere yarns, the fibrous photoanodes sensitization, the electrolyte deposition, the counter electrode production, the DSSF assembly.

WP4. MIAO processes to develop filters for nanoparticles

Start month: 6 End month: 36

Objectives

To develop 3-D porous matrices obtained through mineralization of self-assembling natural polymers (e.g. chitosan, cellulose, gelatine, fibroin) by means of apatite-like phases to be employed as protective devices for selective filtration of nano-particles. In addition the filters will be able to be activated under a magnetic field, increasing the capture efficiency by magnetic and thermophoretic interference as well as they will be endowed with anti-bacterial/ bacteriostatic effect.

Participants: POLLUTION, CNR, Univleeds, FSU Jena, IWNiRZ, UoY

Progress of WP4

- Wool and alginate resulted suitable to form 3D structure by MIAO, exhibiting tubular porosity and interspersed sub-structures hindering the passage of particles. To improve the nanoparticles capture efficiency of filters, polymeric nanofibrous structure was spun on top of the porous sample. The electrospinning technique, demonstrate to be suitable to spun both, synthetic polymers PCL/PHB and PLLA and natural polymer in order to create a tight network of micro-/nano-fibres with filtrating and bacteriostatic potential. As a general concept, it was decided that the part of the filter that will have to exchange moisture with the environment will be made of a hydrophilic and a hydrophobic part. In this respect, chitosan is an hydrophobic material also suitable as a mechanical support, to prevent the collapse of the filter in various environments. Gelatin is a hydrophilic material that exhibits a high potential of exchanging moisture. Composite matrices with different chitosan/gelatin ratios, polymer concentration, and different cross-linking extent were developed and tested by means of capability of aerosol filtration, including collection efficiency and pressure drop, the ability to capture and release humidity, in respect to the development of the new HME devices. Superparamagnetic fibrous constructs were developed by electrospinning of acetate cellulose

incorporating iron oxide nanoparticles (i.e. magnetite, maghemite). The evaluation of superparamagnetic FeHA and magnetite was carried out, with respect to the thermophoretic effect to be exhibited by the new filtering devices.

- Cellulose acetate-based filtering materials were developed by electrospinning (FSUJ), to obtain multi-layered filters with a high permeable substrate material. For this attempt, micro-fibrous CA fleeces (average fibre diameter ≈ 2.5 µm) were produced and stacked with nano-fibrous ones (average fibre diameter ≈ 0.5 µm). The performed air filtration tests showed remarkably increased quality factors for the multilayer fleeces. The quality factor was further increased by using decreased CA concentrations, thus reaching values of about 0.040 Pa^{-1}, which is much higher than the quality factor for commercial available high-efficiency particulate arresting filters (HEPA) of 0.020 Pa^{-1}. Higher volumes of spinning solution or substrate materials with smaller mesh were used during the production process to minimize deviations in the filtration efficiency.

Mineralization of gelatin with a superparamagnetic phase was carried out (ISTEC-CNR) to create the filter's core with a good mechanical properties and a good magnetic interference effect. In particular, carded wool fibres were subjected to mineralization with FeHA to create a non-homogeneous magnetic interference that could increase the diffusive motions of the particles improving the capture. However the yield of mineralization resulted insufficient to generate magnetic fields sufficiently strong for achieving thermophoretic effects.

- Various compositions of silica coated iron oxide Co-LAVA nanoparticles were synthesized and analysed in relation to their hyperthermia properties (FSUJ), thus obtaining, for batches of particles with low saturation magnetisation increased their temperature much more than superparamagnetic magnetite powders.
- A gelatin-alginate matrix was developed with sufficient physical stability to be incorporated into a gelatin-chitosan matrix (ISTEC-CNR), with the purpose to obtain a composite with ability of moisture capturing and also with thermal and thermophoretic properties, for enhanced filtration effects, thanks to the mineralization with a superparamagnetic phase. The approach based on MIAO process was successful, thus reaching a degree of mineralization of about 50% and, thanks to the genipin cross-linking, also adequate physical stability. The previously insufficient hyperthermia effect was greatly enhanced by incorporation of the alginate mineralized with FeHA, so that the final device resulted in a stable and well integrated material. However, in spite of the raise of temperature inside the sample, the hyperthemia effect and the thermophoretic force of these materials, assessed at standard conditions, were found to be too low to induce thermophoretic effects useful to improve nanoparticles filtration.
- Gelatin-chitosan matrices enriched with mineralized wool fibres were developed (ISTEC-CNR), thus finding an improvement in the dispersion of the filtering properties. Therefore, activity was dedicated to the optimization of the composition based on mineralized wool. Despite the bio-mineralization of the FeHA/wool is not an efficient process, these mineralized fibers were well integrated into the filters and the final device was stable.

• A huge activity was dedicated to the assessment of the new HMEs in comparison with commercial devices and electret filters (ISAC-CNR, POLLUTION). On this basis, optimization of the most promising materials produced in the project was carried out, also considering the possibility of scale-up. Among the various techniques explored in the project, electrospinning has been particularly investigated. The key parameters related to the various materials studied in the project were identified, particularly spinning deposition time and the nature of additives and the substrate. Multi-layering is of particular interest, as it is possible to increase the efficiency of filtration, but it resulted not suitable for every type of substrate. To create filters with antibacterial activity and reduced pressure drop, quaternary ammonium-based ionic liquids were used as additive to base compositions of PLA/PHB in concentration of 0.5%, selected to achieve adequate pressure drops, in association with convenient filter thickness that could be managed by adjusting the deposition time. As these materials have properties comparable with commercial EPA filters, the functionalized nanofibers developed in SMILEY are promising for antimicrobial air filtration while their biodegradable properties make them an ‘‘eco-friendly” material. The spinning of cellulose acetate in multi-layer configuration also resulted in filters with high quality factor, however the best results were obtained when a single layer was deposited on nylon grids, thus providing a more convenient method of synthesis. The method is also promising by means of scaling up that was shown to be feasible up to an A4 size. In this respect, the conception and optimization of a new spinning machine should be made.

• Gelatin-chitosan fibres, considered as the most promising matrix for filtration purposes, were investigated in detail and optimized (ISTEC-CNR, ISAC-CNR, POLLUTION). The optimized composition is: hydrogel concentration: 2% w/v; gelatin/chitosan ratio: 70/30; genipin: 2% wt; the freeze-drying process was carried out starting from 40°C with slow freeze-drying. Wool microfibers mineralized with Mg-HA were added to improve filtration efficiency by development of fibres crossing the pores; silver iodide was added to the starting formulation to improve the ice-templating process by freeze drying. Silver iodide showed to yield larger pores that contributed to reduce the pressure drop. A test of scaling up of the process was carried out by repeating the synthesis in a commercial freeze drier. The pressure drop resulted too high for practical uses in both cases; however, homogenous structure and a suitable pores distribution could be obtained in the samples obtained by the lab freeze drier. In respect to these variations in the process parameters, the alignment of the channel pores resulted the most relevant parameter. Rounded and squared-shape moulds were used; this latter ensure a better filling of the freeze-drier chamber, thus being an advantage from the perspective of scalability. Therefore, it was observed that by using an industrial freeze-dryer is possible to develop filters with the same properties obtained with the lab scale equipment with faster production cycle. Unfortunately the addition of mineralized fibres did not result in the establishment of fibres into the pores, which would have significantly arisen the filtration efficiency.

• The improvement of the filtration efficiency induced by thermophoretic effects was assessed in a series of different materials (ISTEC-CNR, FSUJ, ISAC-CNR, POLLUTION). Gelatin-chitosan structures enriched with mineralized wool fibres showed no thermophoretic effect, possibly due to the reduced mass amount of the magnetically active particles in the mineralized structure. In core-shell structures, designed in WP2 and

here developed for HMEs, no relevant changes were detected, by using applied magnetic fields. Electrospun membranes containing LAVA particles exhibited increasing thermophoretic effect in respect to the mineral amount. In conclusion, no relevant thermophoretic effect was detected with the developed 3-D samples. To increase the temperature gradient, a much higher amount of super-paramagnetic nanoparticles should be envisaged. However, this action seems not compatible with the selected manufacturing methods and the potential commercial application (HME filter).

I) electrospinning process provides several limitations in the choice of the "LAVA particles" amount due a problem of coagulation.

II) the MIAO process used to develop the gelatin:chitosan-based samples added with mineralized wool fibres was limited by the impossibility to cover all the wool fibre surface with the mineral phase, and by the maximum amount of fibres that could be added, which was not sufficient to provide thermophoretic effect.

III) all the 3-D structures obtained with this approach showed pressure drop resistances higher than the limit imposed by the standard ISO 9360.

- No particle were released by the various experimented materials during the typical working conditions of a HME device (ISAC-CNR, POLLUTION). Some concerns arise however for biodegradable samples such as PLA/PHB/QAC near the melting temperature (between 50-60°C) and Gelatin/Chitosan -with and without the wool mineralised fibres- for temperature >80°C.
- The mechanical strength of the new materials was tested in extreme conditions (POLLUTION). Gelatin:chitosan constructs were tested after 24 hours of no-stop exposure to high relative humidity passing through, revealing very good stability. Electrospun membranes were tested under high temperature, thus revealing limiting conditions. A number of samples were successfully tested under the gamma ray sterilization process at standard conditions to verify the stability of the filtration efficiency before and after the method proposed for the aseptic production of filters.
- Optimization of the cross-linking procedures of the best composition of gelatin/chitosan-based hydrogel was carried out (ISTEC-CNR). It was found that, as the 3D pore structure was not significantly affected by extensive cross-linking, adequate physical stabilization could be reached, possibly improving also the moisture exchange ability without penalizing the pressure drop.
- The freeze-drying process was optimized by means of heating/cooling cycles and by the testing of various moulds with different thermal conductivity. Modifications of the filter structure induced by the mould were found, particularly by means of uniformity and maintenance of the original shape (ISTEC-CNR, ISAC-CNR, POLLUTION). Silicon and copper were found to be the most adequate materials for the mould; however, as silicon resulted the most expensive, copper was finally elected as the best-in-class material.
- The antibacterial properties to the gelatin:chitosan filters were tested (POLLUTION), both as developed and added with a natural antibacterial (Manuka oil). In spite the use of oil wasnot successful, an intrinsic bacteriostatic and bactericidal effect of the filter was observed, due to the presence of chitosan. The extensive characterization of the functional properties of the new HMEs reported the good properties of the final selected samples, thus confirming the feasibility of the freeze-drying process in a pilot-scale plant with

the polymeric blend developed by MIAO process during the SMILEY project. However, the industrialization process should still be optimized. Particularly, the process generated different porosities in the two opposite sides that penalize the pressure drop. It was confirmed that the removal of one side of the filter, characterized by reduced porosity induced by the freeze-drying process, could improve a lot the pressure drop, reaching the values required by the International Standards. The humidification properties were found to depend, as expected, on the thickness of the filter. To meet the need of enhanced performance, the dead space of the filter was considered for optimization. Particularly the sample holder was designed in 2- and 3-ways configuration by 3D printing, and adapted for minimization of the CO_2 concentration inside the filter. Finally, a design with 3-way configuration was obtained and is now under patenting. The management of medical gases by the new filters was investigated, in respect to what indicated in the International Standards.

WP5. MIAO processes to develop scaffolds for dental regeneration

Start month: 6 End month: 36

Objective

To develop bio-hybrid composites made of self-assembling natural polymers (e.g. collagen, alginate, chitosan, cellulose) mineralized with biomimetic hydroxyapatite (HA) nano-particles. The MIAO process will be addressed to obtain bioactive and cell-conductive scaffolds for the repair and regeneration of human hard tissues such as alveolar bone, cementum and dentin.

Participants: CNR, Univleeds, FSU Jena, IWNiRZ, FINCER, LEMI

Progress of WP5

New polymeric blends able to mediate biomineralization were developed. In particular, collagen, gelatin, alginate, bacterial cellulose and chitosan were used and blended in different concentrations to create composite matrices that induced mineralization with biomimetic HA nanoparticles. BDDGE and Genipin were used as cross-linking agents. The effect of crosslinking on the physical stability of the new blends was investigated. Among the investigated materials, collagen, blended collagen/chitosan and blended collagen/BNC matrices resulted particularly suitable to sustain mineralization without depleting mechanical strength. Morphological and mechanical evaluation of various mineralized composites was carried out to assess the best composition to mimic dental tissues. The most relevant materials were tested by means of cytotoxicity assessment, using well established cell lines. Among 23 tested compositions, 9 were found cytotoxic, particularly among the ones based on flax, 5 showed a decrease of mitochondrial activity without inhibition of cell growth; the remaining compositions, mainly based on fibroin or alginate, demonstrated good cytocompatibility. In some cases the cytocompatible compositions were also studied by using human periodontal ligament fibroblasts.

The new developed biopolymeric blends able to mediate biomineralization, namely collagen, gelatin, alginate and cellulose acetate (CA) were used and blended in different concentrations to create composite

matrices that induced mineralization with biomimetic HA nanoparticles. BDDGE, Ribose and calcium chloride were used as cross-linking agents. The effect of crosslinking on the physical stability of the new blends was investigated. Chemic-physical, morphological and mechanical evaluation of the hybrid materials were carried out to assess the best composition to mimic dental tissues. In particular: i) mineralized gelatin and alginate were used to synthesize highly mineralized tubular-shaped constructs mimicking dentine; ii) highly reticulated collagen membranes were engineered to develop scaffolds for the periodontal ligament; iii) collagen-based matrices were used for bone-like mineralization to achieve scaffolds regenerating cementum and alveolar bone. It was started the collection of information relevant to define the essential requirements in agreement with the international directives for medical and borderline devices. This preliminary activity will serve as a starting point to assess and monitor the associated risks. The process flow charts have been defined analyzing the equipment, raw material and in process controls. The product specification has been draft for both the prototypes related to dentin and tri-layer periodontal scaffold. Once defined the intended use of the foreseen products, a preliminary study on international standards and EU directives dedicated on medical device was carried out in order to define the documentation the product need to have to demonstrate the safety and regularity of the product itself.

- After a deep survey of the chemico-physical and structural properties of the different tissues of the periodontium, the synthesis approaches was to develop fibrous layers, specifically mimicking in thickness and chemical composition the alveolar bone, the periodontal ligament and the cementum, and integrate them into a multifunctional construct (ISTEC-CNR, FSUJ). In order to synthesize scaffolds mimicking the different dental tissues, different approaches of biomineralization were applied to different polymeric matrices (e.g. collagen, gelatin, alginate, CA). In order to guarantee the stability of the hybrid scaffolds in physiological condition, different cross-linking agents (BDDGE, Genipin, Ribose, $CaCl_2$) were evaluated. To mimic the periodontal ligament, membranes obtained by tape-casting process and based on collagen treated with different amounts and type of cross-linker (BDDGE, Genipin, Ribose) were developed and characterized.
- To synthesize a dentin like scaffold, gelatin mineralized with magnesium-doped hydroxyapatite and blended with alginate was used (ISTEC-CNR). In spite of a very good mimicry of the dentin composition, the tubular structure obtained by controlled freeze drying resulted too wide and with improper alignment. Also, the stability of the scaffold in aqueous media did not result adequate for practical uses in vivo. Therefore, it has been attempted to freeze-dry the hybrid composites on hydrophilic/hydrophobic glass slides (MHT/PDT) made by UnivLeeds in order to obtain smaller channels and a more stable material. The mineral phase (mineralization extent ~70 wt%) resulted uniformly distributed in the channel-like porous structure. Moreover the scaffolds produced after the synthesis optimization showed low degradation rate in physiological conditions. The stiffness of the scaffolds was between 30 and 45 kPa/m and a storage modulus between 0.5 and 0.8 MPa depending on the loading frequency, and only little deformations were detected. The cytotoxicity and differentiation potential were evaluated by in vitro test.
- The MIAO process to develop biomimetic hybrid scaffolds made of Mg-doped hydroxyapatite (MgHA)

nanocrystals nucleated on self-assembling collagen fibers were combined with a new cross-linker, Ribose, in order to improve its stability in physiological conditions and limit the cytotoxicity of the scaffolds mimicking the alveolar bone and cementum tissues (ISTEC-CNR). Stable and homogeneous 3D hybrid scaffolds were obtained, with good wettability and ability to preserve its shape in wet conditions.

- The development of dentin-like structures with channeled porosity was also attempted (UnivLeeds) by developing substrates decorated with self-assembling hydrophobic/hydrophilic monolayers obtained by photolithography that gave rise to a grid pattern with hydrophilic properties, suitable for inducing preferential ice nucleation during freeze drying of polymeric hydrogels. Particularly, alginate was selected to create scaffolds with 5 μm wide oriented porosity. Then, mineralization was performed by an approach based on mineral phase formation during freeze-drying. The chemical reaction method yielded mineralisation (ca. 15 wt%) and provided actual embedding of the mineral phase into the alginate fibres with higher resistance to dissolution. Cell growth experiments were performed on the final scaffolds.
- Mineralized fibrous scaffolds were also prepared by incorporation of HA particles into cellulose acetate-based fleeces obtained by electrospinning (FSUJ). The produced CA-HAp fleeces have 1-2 micron fibres and an open and permeable network structure with favorable pore sizes and fiber diameters for osteogenic cells. The added HAp particles have a higher tendency to be located at the fiber surface, that was shown to enhance the biocompatibility and bioactivity of the loaded fleeces, as detected by increase in cells viability in cell culture tests.
- Alveolar bone-mimicking scaffolds mineralized with superparamagnetic FeHA were developed by MIAO process (ISTEC-CNR). Superparamagnetic scaffolds mimicking cementum were instead obtained as electrospun membrane of cellulose acetate added with FeHA powder. In the MIAO process, the synthesis temperature affected the chemical−physical features of the mineral phase of the composites influencing degradability, microstructure, and the magnetization values of the entire scaffold and its biological performance. In vitro investigations indicated the biocompatibility of the materials and that the magnetization of the super-paramagnetic scaffolds, induced applying an external static magnetic field, improved cell proliferation in comparison to the nonmagnetic scaffold. The influence of scaffold properties on cells was preliminary evaluated by seeding human osteoblast-like cells on magnetic and nonmagnetic materials, and differences in terms of viability, adhesion, and proliferation were studied. In the cementum scaffolds, the fleeces showed a fibrous character and a high amount of incorporated particle agglomerates that can be prevented by increasing the amount of matrix material. The optimized scaffolds exhibited very close mimicry of the cementum structure, a pore morphology enabling cell migration, and bioactive composition. The scaffolds showed a moderate biocompatibility comparable to other samples produced in WP5. The magnetic properties revealed superparamagnetic behaviour due to FeHA incorporation.
- Tri-layer periodontal scaffolds with superparamagnetic alveolar bone were prepared by engineering the three different tissue-like layers (ISTEC-CNR), by combining tape-casting and freeze-drying processes useful to induce a stable adhesion within the single layers and generate a 3D functional scaffold..

- Tri-layer scaffold with superparamagnetic alveolar bone and cementum were also prepared (ISTEC-CNR, FSUJ), by piling up the three layers with the FeHA/Collagen at the bottom, collagen in the middle and the FeHA/CA at the top.

In the new superparamagnetic hybrid tri-layered scaffold each single layer respects most of the main characteristic of the natural tissue: the alveolar bone-like scaffold is highly porous with an open and interconnected porosity ranging between 20-500µm suitable to allow the cell colonization and the vascularization, the periodontal ligament-like scaffold is thin and porous and the cementum-like scaffold is massive and not porous.

- A robust characterization of the chemico-physical, morphological, structural, ultrastructural and mechanical features of the raw materials and the various scaffolds enabled iterative optimization of the MIAO process and of other synthesis techniques such as electrospinning that permitted the synthesis of biomimetic fibrous constructs (ISTEC-CNR, UoY, FSUJ). Characterization of the magnetic properties enabled to assess the feasibility to develop biomimetic constructs with superparamagnetic features, thanks to the mineralization/incorporation of FeHA nanoparticles, synthesized by ISTEC-CNR. It has been confirmed that MIAO process could be directed with several macromolecular matrices and different mineral phases. The activation of control mechanisms directing MIAO processes was assessed by ultrastructural analysis, and first conclusions on the effect of the organic/inorganic interaction at the molecular level on the mechanical properties of hybrid composites were made. Hybrid composites with superparamagnetic properties were developed, demonstrating also the need to have sufficient mineral mass to achieve adequate responsiveness to external magnetic signals. In this respect, the use of FeHA powders is more promising. On the other hand, the controlled formation of a small amount of magnetite can significantly raise the magnetic properties of the new hybrid constructs.
- The activity (LEMI) was based on cytotoxicity study of starting materials, or intermediate products in order to eliminate any toxic material at an early stage of the project, and cytotoxicity evaluation of final scaffolds. The *in vitro* cytocompatibility and efficiency of the proposed scaffolds was studied: various preparations were taken into account and tested in order to select the most interesting one (s). Pre-clinical testing using *in vitro* biocompatibility assessment in the framework of GLP were done on samples provided by ISTEC-CNR, FSU Jena, Univleeds and IWNiRZ.

First part: Cytotoxicity testing

Among the 14 materials tested 10 materials were found cytotoxic using the liquid extract protocol with percentages of cell mortality between 38% and 57% and non-cytotoxic using direct contact protocol (except for SMILEY-CA/FeHAp/nylon-FSUJ-02042015 sample for which direct contact protocol has not been performed).

This discrepancy could be explained by the release of some residues not eliminated during the manufacture of the samples.

For all test materials, one can assume that the cytotoxic effect observed can disappear by using the rinse procedure mimicking *in vivo* conditions. The results of biocompatibility tests (see second part) should determine the success potential of an *in vivo* implantation.

Remarks: Cytotoxicity testing could not be carried out using a human cell reactive system (Human periodontal ligament fibroblasts), the sample quantities were not sufficient.

Second part: Cytocompatibility assessment

The goal was to demonstrate the biological acceptance of the material and evaluate the most suitable in terms of cytocompatibility, by means of cell adhesion, growth, differentiation and elaboration of a specific extracellular matrix (ECM). Adhesion of cells to the material plays a fundamental role in regulating ECM synthesis. The physicochemical characteristics of a materal surface regulate serum protein adsorption, and therefore cell adhesion, growth and phenotype expression.

General conclusion: During this period, the cytotoxicity of test materials was tested. None of results obtained show strong cytotoxic effect which would not be compatible with biocompatibility and *in vivo* implantation. The first cytocompatibility study, i.e adhesion and proliferation described in this report, highlights 6 test materials:

The Phenotype expression evaluation allowed to demonstrate that both materials SMILEY - Coll+rib - ISTEC-CNR - 040315 LOT: 04.03.2015 (test material n° 18) and SMILEY-CA/FeHAp/nylon-FSUJ-02042015 (test material n°19) are able to induce differentiation of periodontal cells towards an osteogenic phenotype.

All results obtained related to the inflammatory potential can be acceptable.

Cytocompatibility point of view, 2 test materials seem to be very good candidates: SMILEY - Coll+rib - ISTEC-CNR - 040315 LOT : 04.03.2015 and SMILEY-CA/FeHAp/nylon-FSUJ-02042015.

- 3D cell culture was performed in porous gelatin scaffolds with oriented microtubules designed for dental regeneration (ISTEC-CNR). In this study gelatin, thermally denatured collagen was investigated as a promising low-cost material to develop scaffolds for hard tissue engineering. Dentin-like scaffolds synthesized using gelatin biomineralized with magnesium-doped hydroxyapatite and blended with alginate (HA/Gel+Alg) were studied. A 3D cell culture was performed with Mesenchymal Stem Cells in order to verify the bioactivity of the proposed scaffolds compared to non-mineralized gelatin scaffolds (Gel+Alg) and commercial collagen-based scaffolds (HA/Coll). 3D cell culture with mMSC showed promising properties of the new scaffolds in term of cell adhesion and colonization. In detail, the chemical composition and the aligned porosity were suitable for a nice cell/material interactions. Attachment phase of cell adhesion occurred rapidly and involved physicochemical linkages between cells and material. Looking at the inner surface of the scaffolds after 7 day of culture, more cells were seen in mineralized scaffolds. Indeed, even if the non-mineralized scaffolds presented a more pervious porosity, the absence of mineralized component limited cell adhesion and consequently sample colonization. On the other side, the more compact structure of mineralized scaffolds inhibited cells ingrowth inside the scaffold. Further investigation was done looking at the cell viability with Live&Dead assay, also confirming that the mineralized component is very important in

3D MSCs adhesion. This preliminary study indicated the low-cost biomineralized gelatin scaffold as promising tool for 3D cell culture in dental regeneration.

WP 6. MIAO Processes to develop fibrous photovoltaic devices

Start month: 1 End month: 36

Objectives

To develop MIAO processes applied to natural fibres (e.g. modified cotton, flax, wool, silk, fibroin) to produce new generation dye-sensitized solar cells and mineral phases exhibiting photo-electronic properties (e.g.: SiO_4/GeO_4 apatitic-like phases, TiO_2, ZnO).

Participants: CNR, UnivLeeds, IWNiRZ, UoY

Progress of WP6

The activity of WP6 was focused on developing MIAO processes as a viable way to produce photo-anode materials for photovoltaic fabrics, replicating at the fibre level the structure of a third generation solar cells (so-called Dye-Sensitized Solar Cells, DSSCs). The first part of the work was devoted on identifying the most suitable materials among several hydroxyapatite compounds doped with different cations. Zinc oxide was considered as well. In this respect several ion-substituted apatite phases were synthesized and tested with conventional DSSCs as a model. Among these, titanium resulted as the most suitable ion substituting calcium and/or phosphate inducing suitable photoelectronic properties and promising for development of fibrous photoanodes. ZnO also resulted as a suitable compound for MIAO-derived DSSCs, with better performances than Ti-HA, as expected. However Ti-HA is suitable for MIAO-directed mineralization and exhibits higher specific surface than ZnO, thus higher efficiency of collection.

In this respect MIAO processes were studied and implemented on matrices of different natural fibres, i.e. flax and wool modified by different approaches, to achieve continuous coating with Ti-apatite nanoparticles. Moreover, MIAO process on natural fibers coated by a conductive layer of polypyrrole were carried out, with the purpose of obtaining 3D hybrid constructs reproducing the configuration of DSSCs. The photoanode material in form of Ti-doped hydroxyapatite powder was analysed in order to assess their optical band gap, electrical conductivity, morphology, specific surface area and dye absorbance. Samples with suitable properties were screen printed on a rigid conductive substrate to obtain a thick layer to be tested as DSSC photo-anode. Afterward complete cells were assembled and their properties were compared with the ones of a conventional TiO_2 DSSCs. The performances of the cells produced using the ZnO semiconductor were, as expected, higher than the ones of TiHA and comparable with similar system in literature. DSSCs based on these photoanodes materials were produced and tested with different dye molecules. The best performances were achieved by using titanium doped apatite (15%molar) sensitized with N719 dye. The adsorption capability of the MIAO fibers was evaluated as well and electrochemical analyses were performed. Modified

Flax matrices resulted the most suitable MIAO photoanodes thanks to their dye adsorption properties and morphologies.

Finally, the most promising photo-anode materials were produced in form of fibrous hybrid composites by MIAO process and integrated on a conductive glass to realize an alternative DSSC architecture (MIAO DSSCs).

- The activities reported in this task were aimed at the development of alternative materials to be used in dye-sensitized solar cells. At the same time, FeHA were studied in order to define their possible application as both photanode or dyes. A large part the work was dedicated to the production and characterization of dye-incorporated ZnO crystals, due to the promising results previously obtained. Finally, also alternative deposition procedure for the photoanode were considered, as electrophoretic deposition (EPD) and PLD process. On the other hand, a deeper characterization of FeHA clarified that this materials, as considered up to now, is not yet suitable for the application as photoanode or dye. Further positive indication were obtained considering the incorporation of organic dye into ZnO crystals, in particular the possibility to insert a wide range of dye was demonstrated. Interesenting preliminary results were obtained by the first trials of EPD deposition of Ti-F-HA layers, which will be developed with further studies.
- The production of an alternative DSSC architecture, based on MIAO derived fibrous hybrid able to substitute the traditional screen-printed semiconductor film were produced. The most promising photoanode materials (TiO_2, ZnO and TiHA15), identified during the first 18 months of the project, were successfully biomineralized (ISTEC-CNR/Bio Group) onto functionalized natural fibers (IWNiRZ) using the MIAO process. In view of their application in rigid DSSC photoanodes, functional characterizations were first performed on the MIAO derived fibers in order to assess the stability of the inorganic coating in operating conditions (CNR-Energy group). Afterwards, effective MIAO derived photoanodes were realized by integrating the fibers onto FTO glasses using a transparent polymer; their properties were then analyzed through electrochemical characterizations (CNR-Energy group). The stability and electrochemical properties of MIAO fibers were analyzed, and rigid MIAO-derived DSSCs were successfully assembled using a solid electrolyte and their photovoltaic properties were determined.
- The production of fiber-shape Dye-Sensitized Solar Cells (DSSFs) based on MIAO fibers for obtaining a real flexible photovoltaic device, was carried out. A hybrid composite based on Polypyrrole coating and MIAO inorganic phase were produced by ISTEC-CNR/Bio Group on the natural fibers provided by IWNiRZ. Moreover, the MIAO process was also modified and applied on Ppy+PyE+DSCH conductive fibers, provided by CNR-ISMAC Institute. Mineralization experiments were successfully carried out on flax, viscose and cashmere yarns coated by Ppy doped with PyE and DCHS. The partial fluorination of the mineral layer brought to a more thick and continuous coating of the fiber, which was tentatively attributed to the affinity between fluorine ions and Ppy surface. The electrochemical properties of these samples were determined and the final DSSFs were realized and tested. An optimization of the realization process related to solid electrolyte (CuI) on fiber substrate was done, and a solid-state DSSF based on MIAO fibers with concentric architecture was realized. The first prototype of this device ready for the real application as

integrated photovoltaic system includes electrical connections made on metal wire on counter electrode side and natural fiber with polypyrrole on photo-anode side. The mineralization of natural fibres with titanium-substituted HA is now subject of patenting, which is in progress.

WP 9: Up-Scaling and Assembly of Lab Test Devices

Start month: 24 End month: 36

Objectives

To assemble the filtering composites obtained by MIAO process into the new fibrous filtering devices.

Participants: CNR, POLLUTION

Progress of WP9

• By the end of the project new materials have been successfully developed and are dedicated to:

- Filtration in the Most Particle Penetration Size (MPPS) range
- Antibacterial/bacteriostatic effect
- Heat and moisture exchange

Concerning the filtering device with thermophoretic effects, it was not feasible to induce thermophoretic forces which could increase the filtration efficiency of the porous structures and electrospun membranes tested during the project.

-In the development of filtering devices efficient in the MPPS range it was noticed that:

- PLA/PHB/QAC membranes are nontoxic, antimicrobial and biodegradable and has showed good mechanical strength, good porosity and capability to retain nanoparticles from the airstream. However, they could not resist to high temperature (more than 50°C).
- CA membranes have showed good mechanical strength, high thermal stability, good porosity and capability to retain nanoparticles from the airstream. However, these membranes do not have antimicrobial properties.

The fabricated functionalized nanofibers have shown a quality factor comparable to commercial EPA filters. Therefore, the fabricated functionalized nanofibers can be applied for efficient air filtration while their biodegradable properties makes them an ‘‘eco-friendly” material.

-In the development of filtering devices with antibacterial/bacteriostatic properties, considering the two lines of development proposed during the project for humidification exchange (gelatine-chitosan cross-linked with genipin) and filtration of nanoparticles in the range of the MPPS (PLA/PHB electrospun membranes containing ionic liquids IL), different experimental tests were carried out to investigate the antibacterial properties of the final samples. These tests were carried out by the department of Experimental and Diagnostic Medicine at the University of Ferrara. According to the type of sample to be tested, different procedures were applied according to International Standards. In brief, results from each test confirmed:

1) the pathogens absence through all the three analysed sample areas of the gelatin-chitosan sample;

2) the good performance in terms of inhibition of microbial growth in time. According to Gram negative and positive bacteria, sample showed good bacteriostatic and bactericidal properties respectively. In addition, the polymer blend exhibited high fungistatic behaviour against fungi of Aspergillus genre and great fungicidal properties towards fungi like candida albicans;

3) the good antibacterial efficacy of the electrospun PLA/PHB membranes, which is directly proportional to the amount of ionic liquids added.

The antibacterial and bacteriostatic properties of the gelatin-chitosan blend and of the PLA/PHB membranes with IL greatly facilitated the design of the composition of the active element. Therefore, the effort could be focused to optimize the final blend composition of gelatin and chitosan and of the electrospun membranes.

-In the development of filtering devices with magnetic and thermophoretic interference effect, a new setup providing an alternating magnetic field (AMF) was constructed at Pollution R&D laboratories, to investigate the effects of the thermal increase and the thermophoretic force induced in the samples developed in the SMILEY project. Different frequencies and magnetic field intensities were considered to investigate the magnetic hyperthermia effects. Despite the significant temperature increase of the tested powders, hyperthermia effects were limited significantly when the correspondent nanoparticles were biomineralized or mixed with polymeric matrixes or electrospun in a fibrous network. Cellulose acetate membranes could be functionalised with "LAVA particles" and electrospun in a disordered fibrous network. However, electrospinning process provides several limitation in the choice of the "LAVA particles" type, due to a problem of coagulation.

-In the development of filtering devices functioning as Heat and Moisture Exchangers (HMEs), a brand new test apparatus was developed combining the most frequently methods employed: hygrometric, which is generally used in vivo, and gravimetric, the one suggested by International Standards. The test apparatus was used to investigate the performance of the bio-inspired materials in terms of pressure drop and humidification exchange. The HME blend composition, as defined by the 18th month of the project, consisted in a mixture of gelatine and chitosan natural polymers cross-linked with genipin. The standard procedure was developed by ISTEC-CNR partner with lab-scale equipment. Experimental results of the final samples showed that these exhibit pressure drop and humidification exchange rates in terms of absolute humidity recovered similar to the best HME devices available in the market. The lab-scale process was further optimized and the first tests in a pilot-scale plant were carried out. It was decided to use 3D printing technologies (Fused Deposition Modelling, Stereolitography) to fully design and produce different prototypes of sample holders and supports in Pollution, in reducing production time and cost. The final sample holders, both cylindrical and squared, were manufactured with Stereolitography technique for a better control of the geometry and the surface properties of the sample holder, such as the roughness.

Conclusions

Relevant results were achieved towards the development of the foreseen new devices by MIAO processes. In particular:

➢ New filtering devices for medical uses, with ability of moisture heating and management, were developed by MIAO process applied to composite macromolecular matrices with specific hydrophilic/hydrophobic properties (WP2, WP4, WP3, WP9). The product development is at an advanced stage, including the design and manufacturing of a new sample-holder able to reduce the dead space and significantly improving the properties of the filter. A TRL = 6 was reached for the new HME devices, which are the closest to the market. Patent applications protecting the development of the active component and the holder of the new product are in progress.

➢ Scaffolds with satisfactory mimicry of dental tissues were developed by MIAO process (WP2, WP3, WP5), particularly 3-layer hybrid scaffolds with tailored porosity and mineralization extent, thus mimicking alveolar bone, periodontal ligament and cementum were successfully synthesized by controlled assembling/mineralization/cross-linking processes and by electrospinning. Superparamagnetic scaffolds were also obtained by heterogeneous nucleation/incorporation of FeHA phase with intrinsic superparamagnetic ability. Scaffolds with oriented tubular porosity mimicking dentine were obtained, with promising properties even though further optimization of the scaffold stability has to be pursued. Extensive in vitro tests assessed the potential of all the developed scaffolds to function adequately in processes of dental tissue regeneration. In this respect more focused in vitro and possibly in vivo tests will be needed. A TRL = 5 was reached for the dental scaffolds, with particular reference to 3-layered scaffolds for periodontal regeneration.

➢ The MIAO process was successfully applied for mineralization of wool and other natural fibres with functional Ti-doped HA phases, specifically developed in the project SMILEY. Fe, Fe/Ti, Fe/Ti/F-doped phases were also developed and tested, showing promising properties for enegy applications (WP1, WP2, WP3, WP6). Therefore, fiber-shape Dye-Sensitized Solar Cells (DSSFs) based on MIAO fibers were manufactured for obtaining a real flexible photovoltaic device. An optimization of the realization process related to solid electrolyte (CuI) on fiber substrate was done, and a solid-state DSSF based on MIAO fibers with concentric architecture was realized. The first prototype of this device ready for the real application as integrated photovoltaic system includes electrical connections made on metal wire on counter electrode side and natural fiber with polypyrrole on photo-anode side. In summary, SMILEY project provided a proof of concept of the feasibility of MIAO process to be translated to the manufacturing of active elements for energy production, and to be incorporated in the multi-step stages of manufacturing of a real flexible functioning DSSC. However, further research is needed to translate these results on the manufacturing of DSSC with competitive properties so to be interesting for marketing. A TRL = 4 was reached for the new fibrous photovoltaics, for which a proof of concept of their functioning and feasibility of development by MIAO process was assessed.

All the Milestones of the project were fulfilled, on the basis of the following highlights.

★ The specifications for the final devices to be developed by MIAO process were achieved, in terms of physico-chemical, morphological and functional requirements.

★ Various ion-substituted apatites were synthesized and characterized to assess their photoelectronic properties. In this respect, titanium-doped hydroxyapatite resulted to possess suitable characteristics to function as photoanodes.

★ Processes for the surface modification of natural raw materials, for adaptation to MIAO process, were tested and selected.

★ Superparamagnetic Fe-HA and magnetite were evaluated, in respect to thermophoretic effects to be exhibited by the new filtering devices.

★ MIAO process were successfully set up to generate hybrid composites with superparamagnetic properties.

★ Suitable materials for the development of the new filters were identified. In particular wool and alginate resulted suitable to form 3D structures by MIAO, exhibiting tubular porosity and interspersed sub-structures hindering the passage of particles.

★ Directed gelation methods and freeze-drying processes were successfully applied to achieve oriented fibrous hybrid structures by MIAO mimicking dentinal tissues.

★ The successful assembly of FIP including MIAO-derived functional components was achieved.

★ The key factors to automatize and connect the relevant steps of MIAO processes to develop the filtering devices, the dental scaffolds and the new fibrous photovoltaic devices were identified.

Impact

SMILEY project was designed with the purpose of providing, for the first time, a flexible technological platform for the extensive production of new smart devices responding to very important needs in the fields of environment, healthcare, and energy production. In spite of the evident differences among these fields of application and, consequently, among the envisaged materials/devices for related applications, a common factor was identified, that led to the establishment and development of the project. The common factor was the MIAO process that consists in the heterogeneous nucleation of nano-apatites onto assembled macromolecular matrices, occurring by a bio-inspired process, namely an assembling/mineralization process that base entirely on the information inherent in the structure of the macromolecular matrix and in the unique properties of apatite phases. In fact, the information contained in bio-polymers such as collagen enable pH-driven assembling and provide sites for heterogeneous nucleation of apatite phases that, as driven by crystal order, grain size and ion composition, can exhibit different functionalities, and enable applications in a very wide range of fields with relevant impact. Therefore, SMILEY was dedicated to the study and selection of natural polymers and their ability to assemble and function as macromolecular matrices driving nucleation of functional apatites. On the other hand, apatite nanophases doped with different ions were investigated for potential effects in term of enhanced bioactivity, antibacterial, thermophoretic, superparamagnetic and photoelectronic properties. By this approach, MIAO process was developed as a unique, flexible tool to be flexibly directed to generate various different materials. In particular:

i) filtering devices for uptake of nanoparticles and as HME devices were built and tested;

ii) scaffolds mimicking dental tissues such as alveolar bone, periodontal ligament, cementum and dentin were developed and tested;
iii) new phases with suitable photoelectronic properties were identified and synthesized; prototypes of fibrous photoanodes were developed and tested.

The base materials and compositions already identified for the final devices were optimized and translated to functioning prototypes of the final device, that at the end of the project reached different levels of TRL. By a parallel activity, new materials were developed and tested, with the purpose to extend the range of materials and processes suitable for the final applications. The success in the development of the final devices and the preliminary assessment for scaling up and standardization, even though the product development suitable for the marketing has still to be completed, validates the main scope of the project, i.e. the feasibility of mass production of smart devices for high impact applications, and by using low cost and environment-friendly raw materials and processes. The project activity led to significant advances towards the setup of functioning prototypes, where the functionality was accurately tested, thus opening to the interest in further activity for product development or confirmation of the assessed potentiality.
The intrinsic control existing in MIAO processes ensured the establishment of highly repeatable, scalable and cost-effective processes. Also, SMILEY assessed the translatability of the project results toward industrial applications in terms of efficient industrial production and investments, regulatory requirements, commercial opportunity. In this respect the new fabrication concepts can be transferred to develop flexible production lines producing new custom-made multi-functional products with high level of automation and high product quality. To meet these requirements the following activities were addressed during the project: **i)** building up an integrated system of parameters to overcome production variability; **ii)** definition and validation of suitable product release criteria, such as non-destructive markers for in-line process monitoring in full compliance with current regulatory requirements; **iii)** definition of methods to test the quality of the foreseen final devices by assessing parameters such as material degradation, resistance and composition. In addition, the aim is also to undertake a preliminary economic assessment of the development & manufacturing costs for the novel breakthrough products alongside an assessment of the resulting revenue streams, thus to clearly indicate how to improve resource efficiency and efficacy.
The activity carried out in this respect aimed at drawing preliminary roadmaps to create new production lines with ability to modify their configuration and products, thus implementing adaptive strategies at low costs, with limited transition times and high degrees of freedom, in order to evolve opportunely and in an economically efficient manner with the new processes and products. Therefore, the new concepts proposed by SMILEY may open wide perspectives for the development of cost-effective and highly flexible processes towards mass fabrication of multi-functional devices with huge impact on the world population. Besides, the use of natural raw materials and low temperature processes provides an important gain also by the perspective of enhanced protection of the environment, therefore implying a limited environmental impact and energy expenditure, as well as limited production of industrial waste. In consequence, the application of

these new concepts will also positively impact on the working environments, i.e. working security and ergonomicity as well as a better man-machine interaction. This will definitely improve the quality of life of workers and strengthen, at the European level, the pivotal principle of knowledge-based employment.

SMILEY involved two companies (Finceramica SpA and Pollution Srl) for the exploitation of the outcomes of SMILEY. In particular, Finceramica SpA have already spent significant effort on obtaining a solid basis for commercial exploitation of MIAO technologies applied to biomedical devices, specifically for the design and clinical testing of innovative bone and osteo-chondral substitutes (RegenOss®and MaioRegen®). The products are indeed already providing some return on the investment, therefore SMILEY resulted an appealing opportunity to explore the possibility of translating the MIAO technologies also to the development of devices for tissue regeneration in the dental field.

As well, Pollution Srl was committed to exploit the new hybrid filters, levering on recent preliminary results that illustrate the feasibility of translating MIAO processes towards the large scale development of innovative high-efficiency multi-functional filtering devices. The very positive results obtained in SMILEY are leading to the development of new HME devices towards a commercial product. Patent applicatiosn are currently running in this respect. Moreover, a new sample-holder guaranteeing improved performance was also developed and is now subject of a patent application, as well.

Concerning fibrous photovoltaic devices, in view of the outstanding leap forward promised by the flexible development of portable DSSC fabrics obtained by suitable addressing of MIAO technologies, the project was addressed to gain a significant proof of concept of their functioning; in this respect, as CNR was the main actor in the development of these new materials for energy production, CNR will investigate the interest of relevant companies in the field in promoting further advancement of the development of the new DSSFs. To date, a patent application is pending for the development of the active elements of the new device, based on titanium-substituted apatite nucleated onto natural fibres.

The success of SMILEY, i.e. directing MIAO processes towards mass manufacturing of specific products represents a significant proof of concept, which can strongly encouraging the development of roadmaps addressing to wider industrial exploitation of complex nanotechnology products produced by bottom-up approaches. In fact MIAO processes can be virtually applied to a much wider range of polymeric matrices and inorganic phases; possibly further new concepts might be ideated and investigated to comply with the need to overcome the deterministic, expensive and little flexible top-down processes currently used in nano/micro-fabrication.

Specific impact

New HME devices for healthcare

The success of SMILEY in blending and engineering MIAO-derived active elements for management of the moisture derived from the patient respiration opens the possibility to develop new HME devices. HME filters are used in artificial ventilation to humidify and warm the cold and dry gases from the pulmonary ventilator. Such filters work as heat exchangers, which accumulate the heat delivered from the patient breath and transfer it in the inhalation phase. These filters accumulate the moisture that arise from the breath and then

condensate because of the reduction of the external temperature and afterwards the moisture will be transferred during inhalation. These filters work at room temperature and can humidify and heat the inlet gas of a few degrees. Generally HME filters are used in anaesthesia and for short treatments, while in intensive therapy (long treatment) the use of steam heated water are preferred, because they are better in terms of performance. However they are more complicated (large dead space variables), more subjects to bacteriological pollution and have higher costs. In Europe alone the use of HME filters is estimated as 30 million pieces. The possibility of heating the filter with electromagnetic fields would allow a greater functionality of the filter in terms of heat and hygrometric exchange and with an instant efficiency. A further function of HME filters, in addition to the properties described above, concerns the antibacterial and antiviral filtering capabilities.

In SMILEY the gelatin-chitosan matrices cross-linked with genipin obtained by MIAO were evaluated by means of hydrophilic/hydrophobic behaviour that enable adequate management of the moisture and antimicrobial effect. Also, the tortuous porosity and the physical stability of the blends enabled adequate capturing of nanoparticles. Experimental results of the final samples showed that these exhibit pressure drop and humidification exchange rates in terms of absolute humidity recovered similar to the best HME devices available in the market. Therefore, the new HME devices are very promising to enter in the market and, in this respect, IP protection and product development at an industrial level are being carried out.

Scaffolds for regeneration of dental tissues

The new dental scaffolds are promising for further investigation and attempts to reach more clinically reflective proof of concept and, possibly, the market. In fact, conclusive restoration of dental tissues is an important clinical need; even if significant improvements in oral health have been achieved in developed countries by preventive oral care, socio-economic impact of oral diseases is still a global problem. There is a well-recognised association between oral diseases and other systemic and chronic diseases, due to common risk factors that are related to lifestyle (i.e. dietary habits, use of tobacco, aging). Moreover oral complications of many systemic diseases, such as diabetes, further compromise patient quality of life being cause of pain and working-days lost. Given the progressive raise in the mean age of the active population within EU, it is reasonable to assume that tooth lost and impaired oral function are expected to increase in many developing countries, which need instead to retain full mobility and chewing capacity even at advanced ages. Indeed, tooth loss is associated with a lower intake of hard-to-chew foods, such as fruits and vegetables with a preference for food rich in cholesterol and saturated fat. The DMFT (decayed, missing and filled permanent teeth) index is a universally accepted measure of dental health that expresses the number of compromised teeth in a patient. According with the U.S. National centre for health statistic report (Trends on oral health status, 2007) the DMFT reaches the average value of 15 compromised teeth for patients above 50 years old. Moreover, the WHO Global Oral Health Data Bank reports that severe periodontitis, which may result in tooth loss, is found in 5-15% of most populations.

Traditional treatments of oral disease are a significant economic burden for developed countries, taking 5-10% of the whole healthcare expenses. Progresses in these fields driven by advanced and innovative clinical

treatments are main goals of modern medicine, as they will significantly extend the quality-of-life years of population, also ensuring patient's faster recovery with minimally invasive solutions and improved functionality of the diseased tissues. Besides, the dental scaffolds developed in SMILEY will make obsolete fillings and dentures, improving the health and well-being of many millions of people worldwide. The global dental biomaterials market was valued at $396.8 million in 2008, and it is forecast to reach $699.7 million by 2015 (Global Market Direct, 2009). Given the impact of new regenerative materials for the tooth, the development of advanced products for regenerative medicine can significantly enhance the competitiveness of EU-based industry. SME and large companies will have an opportunity to strengthen their IP and product portfolios by penetrating and extending their shares in the dental biomaterial market.

Flexible fibrous DSSCs

SMILEY project has provided a preliminary proof of concept of the feasibility of building DSSFs by the aid of MIAO processes generating active photoelectronic elements. These new devices, in case of further progress leading to the market, may represent a pioneering attempt to generate devices for energy production that can be directly incorporated in garments, tents/curtains, and in any other support that, when exposed to the sunlight can produce amounts of energy suitable for small devices, that are becoming a determining part of the anyone's life. Generally, the climate change, the decreasing of petroleum supplies and the abrupt increase of the energy demand due to the emerging countries and to an energy-hungry society, has driven the interest towards new energy sources and more efficient devices of energy production. Only a strong acceleration of alternative devices of energy production and an increase of renewables can succeed in reducing pollution, improving the climate and at the same time assuring the energetic autonomy and competitiveness. The current needs of the last decade has put the energy policy not only to replace the KW or MW production plants but also to harness the energy needed to put in operation the huge amount of different electronic devices that are part of the everyday life. Nowadays in fact, consumers all over the world make ubiquitous use of entertainment (e.g.: mp3 players, DVD players) as well as business related devices (e.g.: PDAs, mobile phones). These electronic devices need a wireless, mobile and sustainable energy supply in order to overcome the problem of batteries running out of power when most urgently needed. In this scenario, photo-electrochemical cells show several economic and environmental advantages compared to the conventional industrial processes. Jackets, coats, backpacks, even T-shirts could provide much larger area for integrated photovoltaics than ever-shrinking portable devices themselves. Design studies on solar cells integrated into clothing have been regularly presented since 2000 both in published articles and in industrial fairs, but although consumers and the clothing industry seem to be very interested in the integration of photovoltaics in garments, the advent of real products in the market has been by now delayed by the limited availability and performance of flexible solar cells. Therefore, the success of MIAO processes established in SMILEY may lead to fibrous integrated DSSCs with high cohesion and fibre flexibility. These characteristics are thought to potentially boost the spreading solar integrated devices in a wide range of application where flexible designs are beneficial (e.g.: garments, curtains, Building Integrated Photovoltaics, greenhouses). Coupled with the commodity scale of fibre and textile technology, in fact, fibre-based

photovoltaic devices could help realize cost-effective, scalable solar energy harvesting able to encounter the customers desires. On top of that, low cost production techniques could lead to a correspondent decreasing of the device cost. Besides, the use of energy devices for personal use will reduce the need of batteries, thus decreasing the electronic waste which today represents a major environmental concern.

Dissemination and Exploitation activities

Several dissemination activities have been successfully carried out, bringing to the publication of 16 scientific articles peer reviewed (published) and 1 publication non-peer reviewed.

The presentations made at International Conferences were in total n. 59, 37 orals and 22 posters. 9 workshops, showcases and seminars have been organized to discuss and display SMILEY progress and results.

The first edition of an international conference, **Materials in Medicine (MiMe)** was held at Faenza, Italy on 8-11th October 2013. The event was addressed to converge excellent scientists worldwide active in the fields of biomaterial development, cell-based biological evaluation and surgeons to provide an interdisciplinary panel of discussion on the recent advances and future perspectives of biomaterials, particularly in Regenerative Medicine. The conference was attended by more than 300 delegates from all the five continents and included several Special Events among which, in particular, the EU Commission Forum where SMILEY and other EU-funded projects focused on tissue regeneration were highlighted and the related Project Coordinators invited to give a brief communication on their project and to hold a discussion providing an overview of the current state- of-the-art of the scientific research in the field of hard tissue regeneration, particularly bone, ligament and teeth.

SMILEY project was disseminated also by means of participation to the **1st Engineering & Upscaling Cluster Workshop and General Assembly**, held in Brussels on 12th February 2014. The Workshop was addressed to highlight the best EU projects, selected among the currently active ones, in terms of new approaches and perspectives for the scaled manufacturing of advanced research products. In this respect SMILEY received acknowledgment as a unique project envisaging the production of smart devices for three radically different applications, by a unique, flexible, synthesis approach.

SMILEY project was also selected, among the currently active EU-funded projects, for participation to a meeting, **4M2020**, held on 9-11 September 2014 in Grenoble (France), and focused on advanced manufacturing processes. The purpose of this workshop was to highlight the current advances in micro/nano-manufacturing and draw roadmaps for future approaches to smart manufacturing.

In 2014 the Smiley project was presented at the "International exhibition for European Medical device Manufacturers (**MEDTEC**)" in Stuttgart (D), highly participated by numerous biotech companies; it was also presented at the workshop "Nanoparticles for magnetic hyperthermia: characterization and applications in Nanomedicine and new Materials" held in Parma (IT).

SMILEY was presented at the workshop "**e-Health 2014** –Innovation and Technology in Hospital operating room" held in Rome (IT), which explored the themes of innovation and technology, management,

clinical risk, system integration in Intensive Care in which the filters developed in the smiley project find applications; Smiley was presented at the "38th International symposium on Capillary Chromatography (ISCC)" an interesting occasion to see possible applications of nanostructured filters on chromatography and gas treatment.


MiMe
Materials in Medicine International Conference

1st edition
October 8 -11, 2013 | Faenza, Italy

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European Commission Forum

EC Projects face to face
Chair: Ezio Andreta, President APRE, Italy
Co-Chair: Martina De Sole, National Contact Point MNP, Agency for the Promotion of European Research

Strategic investments in bio materials underpin innovation across existing and emerging sectors. Focusing on Bone Regeneration and the use of bio materials for scaffolding , devices and prosthesis development, the European Commission Forum is the space where new visions, innovative approaches and innovative researches will be related to new perspectives of Horizon 2020.

Mission of European Commission Forum is highlight the central position of the biomaterial discipline in fostering innovation in medical research, looking at past practices and possible developments. The forum will be chaired by Ezio Andreta, President of the Agency for the Promotion of European research and will involve Coordinators of funded FP7 projects in Bone Regeneration.

The Forum, which aims to assess the current status of the innovative approaches and technologies in Regenerative Medicine, referred to the specific field of bone and cartilage regeneration, and on how the synergy among the different Themes (NMP, Health) will provide huge benefits for translational medicine.

Main topic of the European Commission Forum will be:
- Horizon 2020: new perspectives
- Presentation of FP7 projects in Bone Regeneration
- Final Round Table

MIME - 1st Edition
October 8 -11, 2013
Faenza, Italy

SUMMARY OF DEADLINES
June 30, 2013:
Submission form "Research Ideas for the Market"
May 31, 2013:
Submission of Abstract Applications for Young Researcher Competitions
June 15, 2013:
Notification of Abstract Acceptance
June 30, 2013:
Registration at reduced rate
July 15, 2013:
Registration of Presenting Authors
October 30, 2013:
Submission of Draft manuscript

EU-FUNDED PROJECTS PARTICIPATING TO THE FORUM

BIO-COMET (FP7-HEALTH-278907)
Bioreactor-based, clinically oriented manufacturing of engineered tissues
Coordinator: I. Martin, Universitaetsspital Basel, Basel, Switzerland

IMCOSS (FP7-SME-310679)
Injectable Medical Ceramics for Bone Repair and Augmentation
Coordinator: R. Goodchild, CERAMISYS LIMITED, Sheffield, United Kingdom

OPHIS (FP7-NMP-246373)
Composite Phenotypic triggers for Bone and Cartilage Repair
Coordinator: A. Tampieri, ISTEC-CNR, Italy

MAGISTER (FP7-NMP-214685)
Magnetic scaffolds for in vivo tissue engineering
Coordinator: V.A. Dediu, ISMN-CNR, Italy

SMILEY (FP7-CP-FP 310637-2)
Smart nano-structured devices hierarchically assembled by bio-mineralization processes
Coordinator: A. Tampieri, ISTEC-CNR, Italy

INNOVABONE (FP7-NMP-263303)
Novel biomimetic strategy for bone regeneration
Coordinator: O. Hoffmann, University of Wien, Wien, Austria

SANOWORK (FP7-NMP4-SL-2012-280716)
Safe Nano Worker Exposure Scenarios
Coordinator: A. L. Costa, ISTEC-CNR, Italy

bio-comet IMCOSS PHI Smiley Sanowork

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In 2015 Smiley was presented at the international exhibition –"the filtration event **FILTECH-2015**"; the 4th Conference of the Italian Magnetism Association (Magnet 2015) held in Bologna aimed at presenting achievement in applied magnetism bringing together public academic, research institutions and companies.
A workshop highlighting the whole activity and the most significant advances achieved in SMILEY was organized by the project Coordinator ISTEC CNR within the NanotechItaly International Conference held

in Bologna at the National Research Council of Italy from the 25th to the 27th of November 2015. The workshop entitled Nature inspires chemical engineers to develop smart nano-devices and the presented abstracts are described in the Deliverable 8.2.

To complement the dissemination activities, the Smiley Project has been presented during the International event, "**MMC conference 2014**" held in Manchester (UK) Combining the very best of the 2012 European Microscopy Congress and the MICROSCIENCE International Conference and Exhibition series, organized by the Royal Microscopical Society.

To complement the dissemination activities, the Smiley project has been presented during the International event, "**21st congress of the European Society of Biomechanics 2015**" held in Prague (PL) with the presentation entitled "Hard and soft matter interface: interaction of collagen and apatite under tensile stress experimental and computational studies".

The Smiley Project has been presented at the "**BIOMINXIII Symposium**" in Granada (SP) with the presentation entitled "Crystalization of citrate-stabilized amorphous calcium phosphate"; at the Goldschmidt 2015 in Prague (PL) with the presentation entitled "Crystallization of citrate-stabilized amorphous calcium phosphate".

The Smiley Project has been presented at the "XIII International Symposium on Biomineralization – **Biomin-XIII**" with the presentation" The growth mechanism of apatite nanocrystals assisted by citrate: relevance to bone biomineralization" Granada (SP); the "**Bioceramics 26**" held in Barcelona (SP), just a few examples that testify that the dissemination of the project was very intense and effective.

In 2015 several meetings have been organized especially between the two industrial partners Pollution, Finceramica, CNR with different private companies in order to implement the technological transfer of the developed devices, as the "meeting with the director of ICU and anesthesia wards at the Italian national health system premise –Ausl of Piacenza to point out the current weaknesses of the devices features; the meeting with R&D director of GVS-Filter technology company about technical aspects to better perform HME devices, which also declared with a letter of intent a positive feed-back and interest to implement further research activities; meeting with Lyopharm company to analyze industrial techniques of production in order to better perform the further scaling up phase, the meeting with Innovanet consortium whose mission is R&D and manufacturing of innovative products, to discuss about future development and commercialization on the filters developed with Smiley project. Different technological transfer networking meeting have been attended as for example the "technology brokerage event" organized in Trieste (Italy).

Several additional scientific national/international meetings and workshops have been held.

Project website address: http://www.istec.cnr.it/index.php/eng/projects/69-projects-in-progress/european-projects-in-progress/585-smart-nanostructured-devices-hierarchically-assembled-by-biomineralization-processes-fp7-nmp-2012-small-6-310637-en

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