SIXTH FRAMEWORK PROGRAMME

Project no.
NMP4-CT-2006-033277

Project acronym:
TEM-PLANT

Project title:
New Bio-ceramization processes applied to vegetable hierarchical structures

Instrument:
STREP, SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

Thematic Priority:
[3]: NANOTECHNOLOGIES AND NANO-SCIENCES, KNOWLEDGE-BASED MULTIFUNCTIONAL MATERIALS AND NEW PRODUCTION PROCESSES AND DEVICES "NMP"

Publishable final activity report

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Project coordinator organisation name: ISTEC

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

TEM-PLANT: New Bio-ceramization processes applied to vegetable hierarchical structures

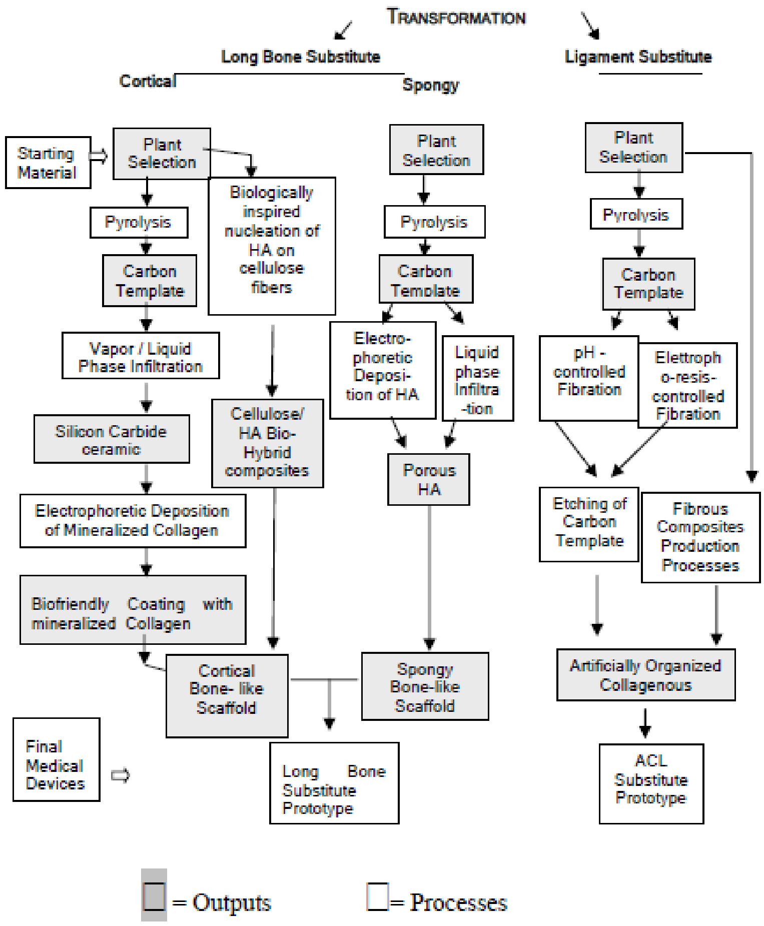
1. PROJECT EXECUTION

1.1 PROJECT OBJECTIVES

The aim of TEM-PLANT was to develop and apply innovative processes to transform hierarchic structures existing in nature into devices with smart anisotropic performances (see scheme below); in particular:

a) **bone substitutes** able to mimic the structure and features of natural load-bearing bone

b) **ligament substitutes,** able to reach the extreme mechanical performances of the natural analogues such as a high number of stress/strain cycles.



1.2 TEM-PLANT PROJECT CONTRACTORS

ISTEC-CNR (Coordinator), Institute of Science and Technology for Ceramics-National Research Council, ITALY

UEN, University of Erlangen-Nuernberg, GERMANY

UNSE, University of Seville, SPAIN

WKP, Wood K Plus, AUSTRIA

IOR, University of Bologna-Rizzoli Orthopaedic Institute, ITALY

FINCER, Finceramica, ITALY

LEMI, Laboratoire d'Evaluation des Matériels Implantables, FRANCE

TUE, Eindhoven University of Technology, THE NETHERLANDS

LTU, Luleå University of Technology, SWEDEN

TEM-PLANT website: http://qportal.istec.cnr.it/lotus/quickr/tem-plant-home

1.3 PROJECT OVERVIEW

TEM-PLANT project focuses on the development and application of breakthrough processes to transform plant-derived hierarchical structures into templates for the exploitation of innovative biomedical devices with smart anisotropic performances and advanced biomechanical characteristics, designed for bone and ligament substitution.

Natural bio-structures usually have properties superior to those of analogous synthetically manufactured materials with similar phase compositions. The remarkable biomechanical properties of bone and ligament tissues depend on their hierarchic structure which is an organized assembly of structural units at increasing size levels. In fact, these structures are highly organized from the molecular to nano-, micro-, and macro-scales, always in a hierarchical manner, with intricate but extremely functional architectures able to constantly adapt to ever changing mechanical needs.

The TEM-PLANT project primary addresses the nano-biotechnologies area and will push the current boundaries of the state-of-the-art in production of hierarchical structured biomaterials. By combining biology, chemistry, materials science, nanotechnology and production technologies, new and complex plant transformation processes will be investigated to copy smart hierarchical structures existing in nature and to develop breakthrough biomaterials that could open the door to a whole new generation of biomedical applications for which no effective solution exists to date.

Starting from suitably selected vegetal raw material, ceramization processes based on pyrolysis will be applied to produce carbon templates, which will be either infiltrated by silicon to produce inert SiC ceramic structures or exchanged by electrophoresis deposition to produce bioresorbable ceramics. For ligament yielding two processes will be developed: pH-controlled and electrophoresis-controlled fibration to generate fibrous collagenous cords with high tensile strength and wear-resistance

Processes for bone substitutes production:

Starting from suitably selected raw materials, ceramization will imply pyrolysis process to yield carbon templates which will be infiltrated by silicon giving SiC ceramic. This inert biocompatible ceramic resembles the cortical structure of human bone. Carbon templates coming from highly porous vegetable structures, will be chemically infiltrated and/or exchanged by electrophoretic deposition with apatitic phases to give spongy bone-like ceramics. The two components will be assembled creating a structural external shell and a bioactive internal core which mimic (in morphology and biomechanics) a long load bearing bone.

Processes for ligament substitutes production

Research activity on ligament would be developed starting from collagen "fibration" in suspension by pH modification on different natural-biological matrices that show a morphologic-structural organization (at nanometric level) with preferential orientation along a specific direction. In this way it would be possible to obtain collagen layers with oriented fibrous deposition that could be totally or partially separated from template matrix via enzymatic or chemical etching. Template matrices will be selected among specialized structures made of lignin and cellulose or biogenic filiform calcifications. To increase the strength and fatigue-resistance of the products, surface modifications and development of fibrous composites is also foreseen.

1.4 STATE-OF-THE-ART

State-of-the-art and limitation of current Bone substitutes

The bone tissue is characterized by a hierarchically organised porous structure (Fig. 1), named spongy bone, enclosed in a denser structure, named cortical bone. The two structures exhibit similar chemical composition but their different architecture makes the cortical part less reactive and mechanically more resistant in physiological environment than the spongy portion, which in turn is able to induce the formation of new bone tissue, due to its high reactivity and interconnected porosity. It is well known that biological materials constantly adapt to the ever mechanical needs. This is achieved by a strain-sensing mechanism; in the case of bone, for instance, some specialized cells (osteocytes) are thought to act as strain sensors and to be at the centre of a feed-back loop, called bone remodelling cycle, where damaged bone is removed and replaced by new material. This process is crucial for the tissue's mechanical adaptation and self- repair and can be activated only in presence of a specialized hierarchic structure.

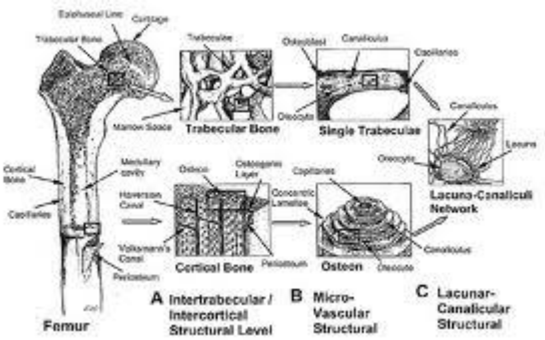


Figure 1. Hierarchical structure of bone.

During the last decades a variety of scaffolds have been developed, and launched on the market, in order to face the increasing need of bone substitutes. Solution ranging from synthetic polymers (e.g. PLA, PGA), homologous or heterologous bone tissues, coral-derived materials up to the more advanced bioceramics or bioceramic-polymers composites have been proposed. Generally speaking, acceptable clinical results have been obtained so far, but no suitable solution have been found yet for regenerating **long and load bearing** bone segments.

The majority of the scaffolds available today have reached a good compromise in term of mimicking the micro and macro-porosity of natural bone. Indeed, open-pore geometries with a highly porous surfaces and microstructures that allows cell in-growth and reorganization and provides the necessary space for neovascularization have been obtained. However, the mechanical strength of a highly porous but “disorganized” scaffold is often insufficient in order to manage the *in vivo* stresses and physiological loadings. Regrettably, the current production processes do not allow to generate an organised hierarchical structure. Therefore, surgeons are now forced to use cadaver bones from tissue banks, just because it can be loaded as soon as they are implanted. As explained above, this is mainly due to the organized hierarchical structure of natural bone that makes the difference to the current synthetic bone substitutes.

State-of-the-art and limitation of current Ligament substitutes

Ligaments, soft collagenous tissues that connect bone with bone, play a significant role in musculoskeletal biomechanics and are another example of the structure-function concept. Again, ligaments have a hierarchical structure that enormously affects their mechanical behaviour. They are constituted of collagen fibrils, a proteoglycan matrix and tenocytes arranged in parallel rows.

Figure 2 shows a ligament which is then split into smaller entities called fascicles. The fascicle contains the basic fibril and the tenocytes. The particular structural crimp and waviness of the fibrils play a significant role in the biofunctional behaviour of ligaments and contribute specifically to the non-linear stress/strain relationship.

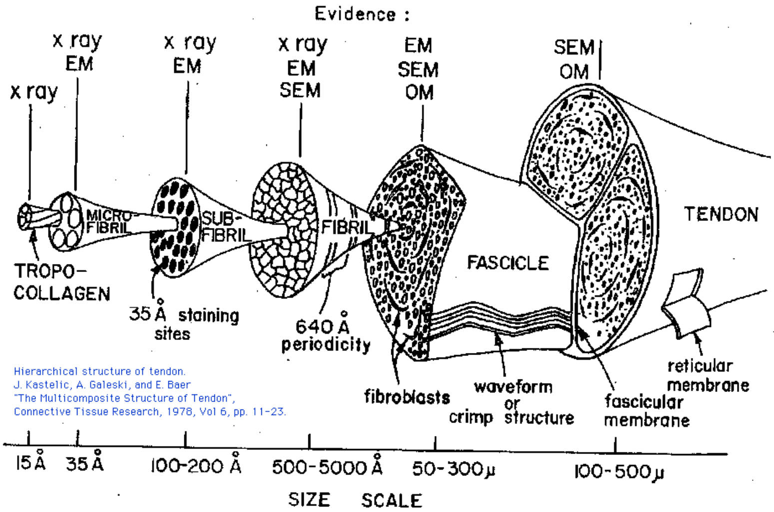


Figure 2. Hierarchical structure of ligament.

Similarly to bone, no solution has been found to substitute natural ligament so far; although significant progress has been made toward understanding the anatomy, composition, biomechanics, and healing of the Anterior Cruciate Ligament (ACL, a very frequently injured ligament) there is still no graft or prosthesis ideally suited for ACL reconstruction.

During the 1970s and 80s, various synthetic materials were designed to act as a permanent ACL replacement device. Hence, the result was invariably a composite of prosthetic material and host collagen and therefore this first generation of ACL prostheses had very poor initial mechanical properties. In fact, these materials did not possess the same biomechanical properties of the native structure and were known to fatigue, stretch, and/or particulate over time. Furthermore, these products did not possess the viscoelastic properties inherent in native tissue, thus making it difficult to accommodate slight discrepancies in graft placement, and also requiring an extraordinarily high degree of skill for precise placement.

Synthetic polymers clinically evaluated for ACL reconstruction include polytetrafluoroethylene (Gore-Tex), polyethylene terephthalate (Dacron; Stryker-Meadox and Leeds-Keio ligaments), carbon fibres (Integraft), and braided polypropylene (Kennedy Ligament Augmentation Device). Although several synthetic ACL prostheses are conditionally approved by the United States Food and Drug Administration (FDA) for salvage cases, or as graft augmentation devices, no prosthesis is unconditionally approved for primary ACL reconstruction. Experimental and clinical ACL reconstruction studies have generally shown poor long-term results due to persistent pain, synovitis, sterile effusions, arthritis, and mechanical breakdown of the synthetic polymers. As a result, currently a torn ACL may either be repaired by means of suturing techniques (done arthroscopically for primary tears, albeit infrequently) or more commonly reconstructed using a tissue graft of autogenous origin, such as the mid-third of the patella tendon or the semi-tendinous-gracilis (hamstring) tendon. While this harvested structure possesses adequate biomechanical strength, it remains a major surgical procedure: an open incision is required to harvest the patellar tendon graft, and even though the joint space is not violated, this surgery is often associated with residual deficits resulting in patellofemoral pathology.

State-of-the-art and limitation of current 3D processing technologies

To date, the development of three dimensional synthetic systems with hierarchical architecture have been limited by the currently available processing technology.

One of the most advanced 3D processing technologies is Rapid Prototyping (e.g sterolithography, laser sintering etc), which allows to generate porous structures with designed geometry out of virtually any material. Porous scaffolds designed in hydroxyapatite with a strut thickness of a few hundred micrometers have been obtained. However the rapid prototyping techniques so far developed have not enough spatial resolution to reproduce the complex hierarchical structure of bone and the resulting scaffolds have very poor bio-mechanical properties.

Other processing techniques based on natural biopolymers are indeed limited by the modest thermal and chemical stabilities of biological molecules.

Preliminary investigation on ceramization processes of natural wood structures has led to the development of the BioSiC technology by one of the project partners (UNSE). This inert biocompatible ceramic is characterized by very good mechanical performances and has a morphology very well resembling the cortical human bone. Parallelly, other partners (UEN, ISTEC) got preliminary results on the development of bioactive, bioreabsorbable porous HA ceramic through electrophoretic deposition and chemical infiltration techniques.

The TEM-PLANT project will start from the knowledge and experience available on those preliminary investigated ceramization processes to step forward on the development of industrially exploitable and economically viable transformation processes. Therefore, by developing material synthesis and assembly processes able to mimic biological principles and hierarchic morphology, we could pave the way for realizing prosthetic devices which could get closer to the extraordinary performance of human tissues. In this respect, native or semi-processed wood and plant habits may be successfully used as templates for generating, through a sequence of pyrolytic, infiltration and/or electrochemical processes, a ceramic or polymeric material that shows uniform as well as non-uniform (hierarchical) pore structures.

Plants include self-supporting taxa that are shrubby or tree-like. In the broadest sense it also includes lianas, vines, root-climbers or grasses. By selecting among the wide variety of native plant structures, advantage can be taken of their **lignocellulosics** cell wall architecture that exhibits hierarchical fibrillar as well as a porous composition (Fig 3).

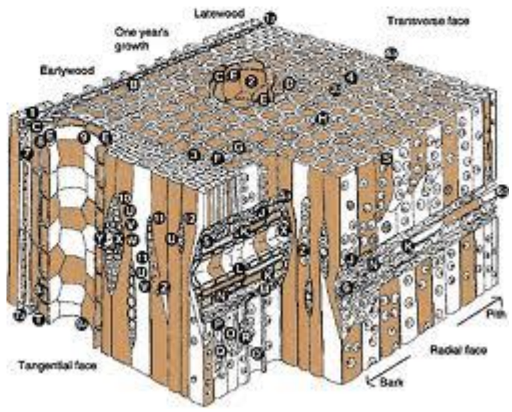


Figure 3. Cellular structure of wood.

1.5 ECONOMIC AND SOCIAL IMPACT

The painful disability of bone and joint related diseases is a major problem of ageing populations in modern societies and its social impact is enormous. Worldwide, the number of skeletal regenerative and reconstructive surgeries is estimated as have increased at annual rate of more than 20% from 1998 to 2004. With such a demand, and considering the performance but also the current limitation of the medical devices available on the market to date, there is a recognized societal and economic need for synthetic materials with appropriate mechanical and biofunctional performance to be used in orthopaedic applications. This need represents a challenging but motivating opportunity for the European scientific and industrial community.

The challenges in developing industrially-viable manufacturing process to produce synthetic materials with adequate mechanical, biomechanical and functional characteristics are great: indeed, only hierarchical, biointeractive, nano- micro- and macro-structured composites materials could aim to reach the performance level of natural skeletal tissues.

TEM-PLANT is involved in a sector with significant **economic impact.**

In 2002, the orthopaedic market amounted to approximately $14 billion and is expected to double to $27 billion in 2006. A few major companies dominate the market – the ten largest companies control 70 % of the market. There is also a number of small and medium-sized players. The US is the single largest market for orthopaedic products and represents approximately 60 % of world market sales. Reconstruction products, the segment related to TEM-PLANT project, is the largest segment within the market for orthopaedic products and represents approximately 50 % of the total sales. During recent years, price increases of up to 5 % per year have been implemented, and this, together with increases in volumes, has contributed to strong growth within the segment. The Consortium believes that reconstruction products made from biomaterials will exhibit a rate of growth that will exceed this level.

Bone substitutes market. The European bone substitutes market for orthopaedic generated $**51.3 million** in 2004. Despite tight hospital budgets and ongoing fears surrounding disease transmission through human and animal derived products, this market is forecast to increase at a compound annual growth rate of 13.1% over the period from 2004 to 2009. Growth in this market is being supported by the clear benefits of bone substitutes relative to autografts, including less time in the operating room, and significantly less pain and recovery time for the patient (e.g. autograft transplantation is associated with an average morbidity rate of 20% of the patient's donor site). On the other hand, allogenic bone grafts have still risks of disease transmission, although the meticulous controls of the human donors and of processing techniques have dramatically increased. A medium-impact market study estimates that use of alloplastic materials has been approximately 35 % in 2004. By 2009, the European BGS market is expected to reach a value of $95 million.

Ligament substitutes market. Ligament surgery is a fast growing operative treatment due to the increased occurrence of sport accident injuries. It has been estimated that there are at least half a million surgical reconstructions of injured ligaments every year in Europe, Japan and USA. Currently a torn ACL is commonly reconstructed using the mid-third of the patella or the semitendinous-gracilis tendon of the injured patient, and this is due to the lack of suitable synthetic ACL substitutes. It is easy to forecast the major economic repercussion of these kinds of surgery both in terms of social impact -when they result in long hospitalization time, requirements for extensive rehabilitation therapy, chronic pain, incomplete recovery of full function or even permanent immobilization of the patient and in terms of industrial opportunities -when the injured tissues require replacement by artificial prostheses. Moreover, the tears to the ACL, a common sports injury of the knee, have received a great deal of media attention since an ACL injury may jeopardize a promising young athlete's future career goals. Since there are essentially no ACL substitutes currently on the market (just few synthetic "old fashion" devices which are experiencing little sales) the actual market is small, but the market potential of a "new generation" of ACL substitutes, which could be proven to be clinically efficient and cost effective, is estimated to exceed 200 € million just in the EU.

1.6 PROJECT DEVELOPMENT

a. SELECTION OF RAW MATERIALS

The selection of suitable raw materials to be transformed in bone and ligament scaffolds was made on the basis of morphological and mechanical specifications for bone and ligament, provided by clinical and industrial partners.

In particular, different types of woods were investigated and transformed in inorganic scaffolds, for bone substitutions. In the same way, fibrous vegetable structures were pyrolysed and it was attempted to build ligament-like scaffolds by using pyrolysed vegetable fibres as templates.
Different techniques were applied to induce suitable modification of morphology and structure of some raw materials to better simulate the pore size and interconnection of bone tissue.
A wide spectrum of characterization techniques was applied, to detect physico-chemical, morphological, structural, ultra-structural and mechanical properties of the developed bone and ligament scaffolds. Moreover, in vitro biological characterization was carried out on selected biomaterials and scaffolds to assess cytocompatibility, bioactivity and regenerative potential, prior to in vivo tests.

b. SCAFFOLD FABRICATION

BONE

The substitution of the cortical part requires a limited porosity and remarkable mechanical strength, whereas the spongy portion should exhibit high porosity and pore interconnection and high bioactivity. In the course of the project, several woods were considered as templates for cortical or spongy-like bone substitute. After the first two years, the most suitable woods to be transformed in the cortical and the spongy portions have been identified and converted in inorganic phases, i.e. biomorphic silicon carbide (BioSiC®) and hydroxyapatite (HA), to substitute cortical and spongy bone respectively. To simulate the cortical part of bone, the transformation of different woods in biomorphic silicon carbide was carried out, by means of a well assessed patented procedure after suitable modification to adapt to different wood structures.
To obtain scaffolds simulating spongy bone, two different processes for transformation of raw materials were developed:
1) a multi-step process allowing to transform native vegetable structures in hydroxyapatite, through a sequence of intermediate biomorphic materials (namely carbon, calcium carbide, calcium oxide, calcium carbonate and finally HA), by maintaining the original structure and mechanical consistency.
2) a sol-gel process aimed at transforming vegetable structures through repeated infiltration of suitable precursors and subsequent heating for elimination of the organic part.
These processes were developed and applied on different woods, until a final decision on the most suitable raw materials was made. Finally Rattan was selected as the most promising wood to simulate the spongy part of bone.

A substitute of the spongy bone, based on mineralized collagen, was also developed. A biologically inspired process was optimized to self-assemble collagen fibrils and to nucleate nanostructured apatite particles on them, so as to obtain a 3d construct simulating the newly formed bone.

After the first two years of project, it was assessed that the most suitable concept to design a biomorphic load bearing scaffold was a bi-component scaffold made of:

i) an outer part (the scaffold "shell") made of bioSiC, derived by wood transformation and characterised by high compression strength and suitable porosity to allow cell attachment and osteointegration.

ii) an inner part (the scaffold "core") made of a highly porous bioactive materials made of either collagen fibres mineralized with biomimetic hydroxyapatite or a biomorphic inorganic scaffold made of HA obtained by a suitable transformation process.

In order to increase the bioactivity of the outer part of the scaffold, a bio-friendly coating was applied. Different methodologies were studied to apply a homogeneous bioactive layer on the bioSiC surface; finally the most suitable technique resulted to be a soaking of the scaffold in suitable solutions containing the ions of interest, namely calcium, phosphate, magnesium, silicon; it was also studied a procedure aimed at activating the SiC surface, to improve the adhesion of the coating layer. Finally, the optimized parts of the scaffold were assembled and implanted in vivo.

The results were very promising: the biomorphic scaffolds in HA obtained by multi-step process, implanted in non-load bearing sites in small animal, exhibited high bioactivity and osteoconductivity; the bi-component scaffolds (bio-friendly coated biomorphic SiC filled with bio-hybrid HA/Collagen composite) were implanted in big animal in load-bearing sites, exhibiting very good osteointegration and bone regeneration.

LIGAMENT

At the beginning of the project, vegetable fibres were selected for transformation in templates for ligament-like scaffolds. It was shown that the pyrolysis process was suitable to completely eliminate the organic part of the fibres, while maintaining the original morphology and micron-size details. Techniques of electrophoresis were investigated to obtain collagen-based scaffolds, starting from vegetable fibres. However, such structures demonstrated not suitable for electrophoresis techniques, due to their cellular structure and the consequent shielding of the electric fields which hampered the collagen deposition onto the fibres.

Then, collagen, cellulose and other natural polymers were investigated and processed to obtain fibrous structures with complex morphology and ligament-like mechanical strength. Tape casting procedures resulted elective techniques to obtain thin membranes of collagen-based materials; the fibrillation of cellulose was carried out to obtain suitable raw materials for further processing. In the second half of the project, a variety of composite fibrous materials were developed, starting from processed collagen, cellulose and other additives, a wide range of characterization techniques was applied to assess the behaviour of raw materials and assembled scaffolds in dry and wet state; on this basis a selection of the most suitable raw materials was made and in many cases good elasticity and suitable mechanical resistance were obtained.

In order to increase the mechanical performances of ligament-like substitutes, a new technique was investigated to obtain cylindrical collagen / cellulose composites with a radial symmetry, with the purpose to develop bi-component ligament-like scaffolds, made of a tension-resistant part and a bioactive core allowing cell colonization and proliferation. Finally, the most suitable compositions and processing methods were selected and prototypes of ligament-like scaffolds were prepared. In particular, scaffold made of stripes of collagen layers, opportunely braided, exhibited good elasticity and capacity to withstand repeated fatigue cycles.

In vivo proof of concept was carried out by implanting some of the prototypes on small animals, in replacement of Achille’s tendons, thus revealing that the new scaffolds were conducive to cell attachment and proliferation and induced the synthesis of collagen and formation of new cartilaginous tissue.

c. CONCLUSIONS

To conclude, very good results were obtained during TEM-PLANT project. In particular:

1) a new concept of bone scaffolding was developed, thus obtaining prototypes of biomorphic scaffolds with a very strong potential to be applied in clinics in the next 5-10 years;

2) preliminary in vivo experiments proved that the development of ligament-like scaffolds based on the processing of natural polymers is a feasible way to obtain regenerative scaffolds for tendons and ligaments;

3) new knowledge was gained in the field of nanotechnology, thus pushing the current boundaries of the state-of-the-art in production of hierarchically structured materials. New knowledge was also gained, concerning chemico-physical phenomena at the basis of the transformation of natural raw materials and of the self-assembling and mineralization processes;

4) the development of biomorphic devices potentially opens the door to new approaches for the achievement of smart materials and devices with exploitation perspectives wider than the biomedical field. An intriguing possibility is that of simultaneously achieving high values of strength and toughness, for which ordinarily there is a trade-off, in addition new materials with extreme values of physical properties such as thermal expansion or piezoelectricity can be obtained.

Materials able to maintain adequate properties at extremely high temperatures and mechanical stress are highly sought after for use in several different applications, including, for example, catalytic silencers, space vehicles, turbine equipment for power generation plants and aircraft engines, like turbine blades, vanes, shrouds, and combustor components, and metal forming and glass blowing equipment. The exploitation of the innovative techniques proposed in TEM-PLANT, based on wood/plant ceramization and self-assembling techniques will allow to overcome the problem of the structural complexity and to reduce the characteristic size of the smallest structural elements, in a wide range of technological applications;

5) the development of biomorphic materials and devices is based on the use of natural raw materials and natural waste to be transformed into complex structured smart devices: this concept meets very important requirements on recycling and environment safeguard.

2. DISSEMINATION AND USE

1) Bi-component biomorphic scaffold

The RTD activity carried out in TEM-PLANT led to the development of an italian patent: **"IMPLANTS FOR "LOAD BEARING" BONE SUBSTITUTIONS HAVING HIERARCHICAL ORGANIZED ARCHITECTURE DERIVING FROM TRANSFORMATION OF VEGETAL STRUCTURES"**, invented by CNR, UEN, UNSE and LEMI, based on a bi-component bone scaffold made of:

a) a cortical-like part, represented by a hollow cylinder in BioSiC, simulating the cortical bone, externally coated with a layer of biomimetic hydroxyapatite (see below, item No 3) or a layer of electro-deposited Type I Collagen bio-mineralized with hydroxyapatite (see below, item No 4);

b) a spongy-like part, represented by a 3D scaffold, characterised by high bioactivity, open porosity and capacity to be progressively resorbed, that simulates the spongy part of bone. It is made of either a 3D bio-hybrid hydroxyapatite/collagen composite (International patent no W02007045954) or a biomorphic hydroxyapatite scaffold (see below, item No 2).

2) Biomorphic wood-derived HA

The RTD activity carried out in TEM-PLANT led to the development of biomorphic scaffolds in hydroxyapatite, derived by the transformation of several woods including rattan. The research was published in: Tampieri A, Sprio S, Ruffini A, Lesci, GI, Roveri N., 2009. *From wood to bone: multi-step process to convert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering*. Journal of Materials Chemistry. 19 (28), 4973-4980.

3) Mineralization process for bone device

The RTD activity carried out in TEM-PLANT led to the development of a procedure for applying an uniform bioactive coating on BioSiC surfaces, by soaking in modified SBF solutions. The invention is part of the patent indicated above (Item no 1).

4) Biofriendly coating of collagen (electrodeposition)

An existing procedure (International Patent no WO2008146113) was used to apply an uniform bioactive coating on BioSiC surfaces, by electro-deposition of Type I Collagen mineralized with hydroxyapatite. The invention is part of the patent indicated above (Item no 1).