Bio-Mechanically Active Ceramic-Polymeric Hybrid Scaffolds for Tissue Engineering

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Abstract. The research develops and tests new hybrid biomimetic materials that work as mechanically stimulating "scaffolds" to promote early regeneration in implanted bone healing phases. A biomimetic nanostructured osteo-conductive material coated apparatus is presented. A bio-inspired approach to materials and template growth of hybrid networks using self-assembled hybrid organic-inorganic interfaces is finalized to extend the use of hybrids in the medical field. Combined in vivo, in vitro and computer aided simulations have been carried out. A new experimental methodology for the identification of design criteria for new innovative prosthetic implant systems is presented. The new implant design minimizes the invasiveness of treatments while improving implant functional integration. A new bioactive ceramic-polymeric hybrid material was used to modify odontostomatological Titanium implants in order to promote early fixation, biomechanical stimulation for improved scaffold mineralization and ossification. It is hybrid ceramic-polymeric nano-composites based on Hydroxyl-Ethyl- Methacrylate polymer (pHEMA) filled with nanosilica particles that have shown biomimetic characteristics. This material swells in presence of aqueous physiological solution leading to the achievement of two biomechanical functions: prosthesis early fixation after and bone growth stimulation. Such multidisciplinary approach explores novel ideas in modelling, design and fabrication of new nanostructured biomaterials with enhanced functionality and improved interaction with OB cells.

Introduction

Innovative tissue engineering biomimetic materials based on hydrophilic polymers show attractive physical, biological and mechanical properties for several biomedical applications [1-6]. Highly biocompatible novel hybrid materials based on fumed silica and hydrophilic poly-(hydroxyl-ethyl-methacrylate) (pHEMA) have been developed by the authors [7]. The addition of fumed silica improved the self-organization of the polymeric network promoting hydrogen bonding of the polymeric chains with the hydrophilic nanoparticles. The resulting nano-composite consisted in more rigid transparent materials with surprisingly improved mechanical strength [7] that overcome one of the major drawbacks in hydrogels applications associated with their poor mechanical properties. Early studies confirmed that the nano-filled hybrid composites possess biomimetic and osteoconductive properties that can be useful in the design of mechanically bioactive innovative scaffolding systems for Osteoblast (OB) growth [7]. In healthy conditions, modelling and remodelling collaborate to obtain a correct shape and function of bones. This condition is completely altered when bone is implanted with a rigid prosthesis [8, 9]. Loads on bones cause bone strains that generate signals that some OB cells can detect and respond to. Threshold ranges of such signals are involved in the control of modelling and remodelling [10-15]. Remodelling processes repair the injury by removing and replacing the damaged tissues with new bone. Moreover, overloading (or under-loading) alters such phenomenon [15]. Early studies by Wolff (1892) stated that mechanics could determine changes in the architecture of bones [13]. In 1964 Frost expressed mathematically the reactions of the bone tissue to given stimuli to quantitatively assess bone deformations [14]. Remodelling processes repair the damage removing and replacing the damaged tissues with new bone. Different studies proved that the micro-damage threshold is about 3000 micro epsilon strains [11, 16].

Adaptive properties of bone, in fact, can benefit of use of biomimetic (biomechanically compatible and bioactive) scaffold biomaterials. The use of biocompatible and biomechanically active interface that can be "designed" to reproduce bone compatible and biomimetic strain distribution is discussed in the present paper.

Materials and Methods

Biomimetic/Biomechanical Approach

Our Biomimetic and Biomechanical approach resulted from a parallel mechanical and physical characterization of new hybrid material coupled to the biomechanical Finite Element analysis of the new system investigated (implanted bones).

Materials

Commercial 2-hydroxyethyl methacrylate was purchased from Sigma-Aldrich Chemicals Co., (St. Louis, MO, USA). Fumed silicon dioxide (Aerosil 300 Degussa, Germany) with a mean diameter of 7 nm and specific surface area of 300 $m^2\ g^{-1}$ was utilized as the bioactive filler. The initiator, **α-α**' azoisobutyrronitrile (AIBN), was purchased from Fluka (Milan, Italy). HEMA monomers were mixed with increasing amount of fumed silica (4 to 30% by volume). The resin was poured in 2 mm thick plane moulds, polymerized in a forced air circulation oven set at 60 °C for 24 hrs and finally post-cured at 90 °C for 1 h.

Sorption Swelling Tests

The planar samples were used for the aqueous isotonic saline (0.15 M NaCl) solution sorption and swelling experiments. The aqueous solution uptakes in the initially dry samples were determined at equilibrium by gravimetric measurements in a 0.1 mg Mettler Toledo balance (Milan, Italy). The advancing swelling fronts in the limiting Case II anomalous sorption [17, 18] of the samples were monitored measuring the thickness of the un-swollen residual glassy core as a function of time. The equilibrium sorption and swelling experiments were performed at 37 °C (thermostatic water bath) until constant weight up-take was monitored (100 h).

Finite Element Analysis (FEA)

The solid models of the odontostomatological implants were generated using Solidwork 2007 software. Titanium implant and the hybrid material screw substituting part were modelled. The FE model was obtained by importing the solid models into ANSYS rel. 9.0 FEM software (Ansys Inc. Houston) using IGES format. The volumes were meshed with tetrahedric elements, resulting in a 3D FE model made up of 31,240 elements and 35,841 nodes. Accuracy of the model was checked by convergence tests [10].

Mechanical Characterization

Shear elastic modulus measurement on dry and swollen p-HEMA Hybrid nano-composite were performed using a METTLER-TOLEDO (Zurich, Switzerland) dynamical mechanical tester operating in shear mode (DMA). The elastic and viscous components of the shear modulus were measured under constant frequency in isothermal condition. The samples were dried under vacuum at a 60 °C for 24 h before testing. In the shear test mode, the 10 mm diameter and 2 mm thickness sample disks are placed between three steel plates forming a symmetrical sandwich. An isothermal scan at 37 °C in a dry Nitrogen purged environment was performed. The deformation control was set to 10 **μ**m and a force limitation of 0, 9 N was applied at an oscillating frequency of 10 Hz.

"In vitro" Evaluation of Implant Primary Stability

The in vitro study was aimed at evaluating the possibility of improving the primary stability of modified oral implants by means of three-dimensional scaffolds in hybrid ceramic-polymeric nanocomposite material.

Both the test and the control fixtures were randomly divided into 4 groups; each test group was made up of 9 implants while each control group consisted of 3 fixtures. At implant placement, insertion torque values ranging between 43.4 and 44.5 and between 44.2 and 45.7 Ncm were recorded in the test and control group, respectively. In groups 1 to 4 and in groups 5 to 8, the implants were removed after 1, 6, 12 and 24 hours. The removal torque values were recorded as previously described [23, 24].

'In vivo' Evaluation of Implants Bioactivity and Osteo-induction

The in vivo study was aimed at evaluating the bioactivity and osteo-inductivity of the hybrid ceramic-polymeric scaffold. Unmodified Titanium dental prostheses, and modified and coated prostheses were implanted in laboratory rabbit's femur and removed after two months. Micro-Computer Tomography has been run on the explanted femurs and bone density and distribution on the implant has been evaluated.

Discussion

Our research was aimed to design a biomimetic dental implant finalized to favour adaptive directionally organized OB growth in implanted mandibular bone. In order to achieve this result, both a proper biomimetic scaffolding material and an external implant screw portion have had to be designed.

The biomimetic characteristic of our hybrid materials have been investigated both for mechanical and swelling properties. Physiological bone material behaviour to be mimicked by the bioactive scaffolding material relates to the following aspects:

- Mechanical properties (in the dry and swollen states)
- Bioactivity (in vivo implant)

Biomimetic Target Mechanical Properties of Hybrid Nano-composite

The dry Hybrid pHEMA nanocomposite with compositions ranging from 4 to 30% by volume of nanosilica were isothermally shear tested in a Dynamic Mechanical Analyser operating under a Nitrogen dry atmosphere at 10Hz and 37 °C. The samples showed a predominantly elastic behaviour (the viscous component was negligible for all compositions). The values of the measured Shear moduli are reported in Fig. 1. The measured shear moduli of pHEMA-Nanosilica composites do not follow classical Halpin-Tsai equation for particulate composites (the upward full line reported in Fig. 1) [20]. A linear dependency of shear modulus values for progressively increasing content of nanosilica loading has been observed, instead. This unexpected behaviour indicates the hybrid nature of the nanosilica pHEMA composites.

In order to define the proper nanofiller/p-HEMA ratio of potentially suitable hybrid nano-composite the target properties requirements are:

- Similar to bone rigidity during implantation (E=6-15 GPa and G=2-4 GPa [19]) in the dry state
- Similar to cartilage and ligament flexibility during osteo-integration (when swollen).

Shear moduli comparable to those of the cortical bone have been measured in the dry state for nanosilica volumetric fractions ranging from 4 to 12%. A volume fraction of 5% has been then chosen for sample preparation and FEA simulations.

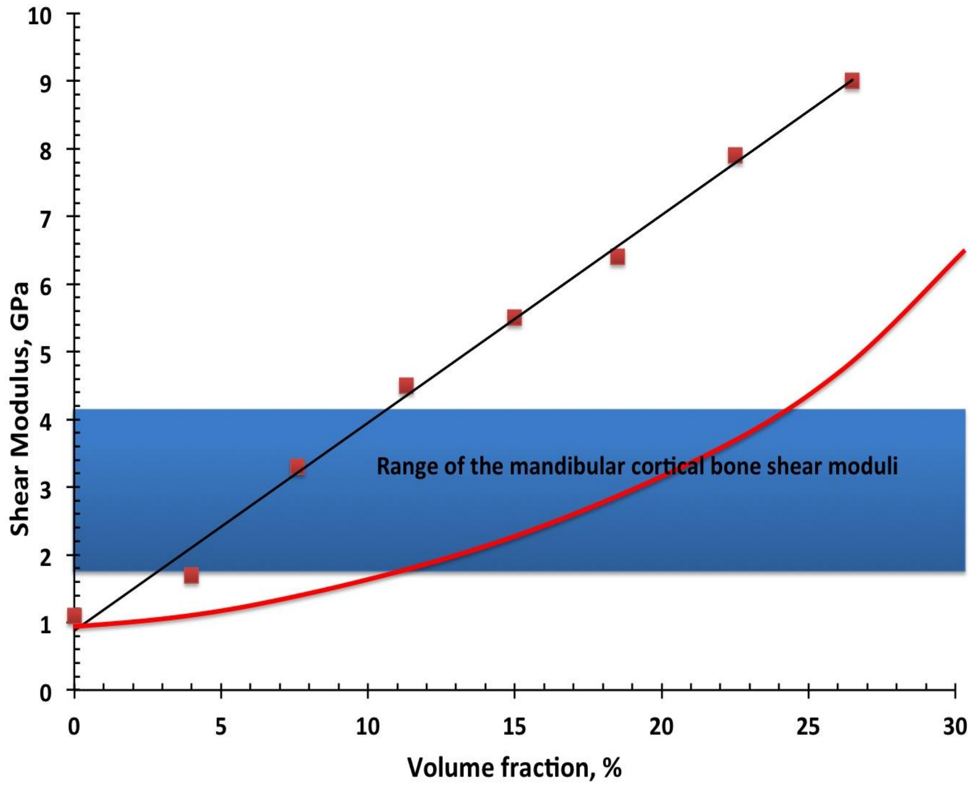


Figure 1. Shear moduli of the hybrid nanocomposites at different nanosilica filler loading. The theoretical Halpin-Tsai curve is reported for comparison in the figure.

Elastic moduli (tensile test) ranging from 2-20 MPa (strain hardening effect) have been measured for the rubbery swollen hybrid composite (5% by volume). This value turns comparable to that of the periodontal ligament strained in the same conditions and to that of an articular cartilage [19, 21].

Swelling Behaviour

The 5% hybrid nano-composite dramatically swells in isotonic water solution (fig. 3) picking up 50% of its dry weight while reducing its shear modulus down to 2-3 MPa (measured in the DMA at 10 Hz). Such phenomenon is associated to the water induced polymer plasticization that reduces the polymer glass transition temperature below the test temperature. The sorption behaviour in a physiological isotonic 0.15 M NaCl solution held at 37℃ of our 5% volume fraction glassy hybrid materials has been investigated for both solution weight uptakes and swelling kinetic. Once exposed to the aqueous solution, the initially dry and glassy pHEMA nanocomposite 2 mm thick plates starts to swell showing a clear front dividing the rubber-swollen outer portion and the unaffected glassy core. The glassy core thickness progressively reduces as the swollen front advance through the sample. A measure of the swelling kinetic, which has been reported in Fig. 2 as a function of the square root of time [22], is given by the rate of reduction of the glassy core as a function of the time. The observed swelling front initially advanced at constant rate according to the limiting relaxation controlled anomalous sorption mechanism indicated as "Case II sorption" [17-19, 22]. Initial linear swelling rate is of about 0.10 mm per hr. As swelling goes further, however, a diffusive resistance develops in the outer swollen skin leading to a diffusion controlled swelling of the remaining glassy core [22]. When swelling fronts meet, sample weight uptake is about 27% but it continues to increase up to the equilibrium value of 40%. This is due to the achievement of a complete equilibrium swelling through the samples thickness. At equilibrium 14.5% increase of the sample thickness and 50% volume increases have been measured. These values have been used to evaluate by Finite Element Analysis simulation the dimensional changes occurring in the complex geometry of the modified dental implant described in the subsequent paragraph.

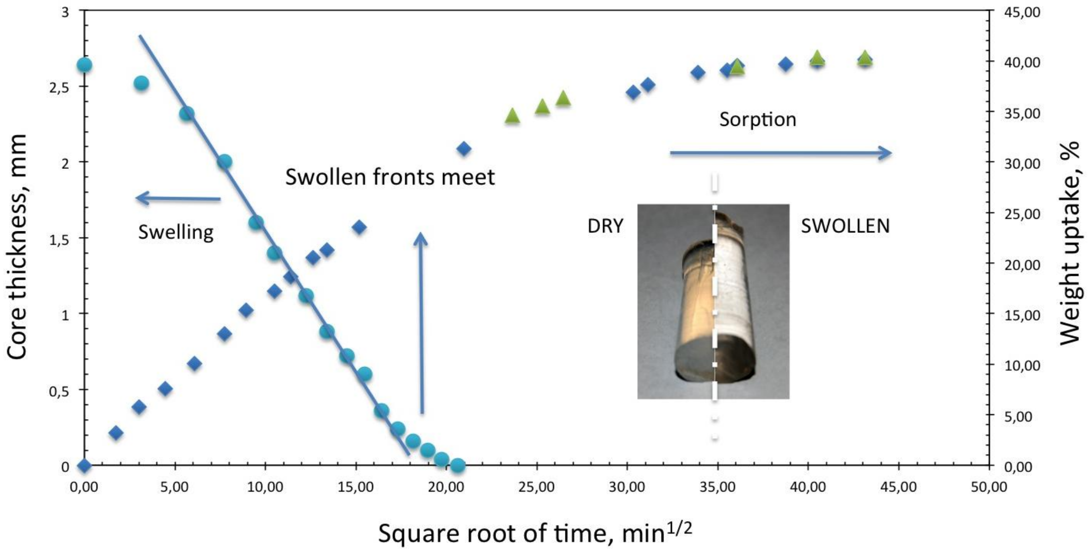


Figure 2. Swelling and sorption kinetic of a 5% by volume hybrid nanocomposite in 0.15M NaCl water solution (isotonic).

Bio-metic Implants for Adaptive Properties of Bone

The use of biocompatible and biomechanically active interface has been "designed" to reproduce bone compatible and biomimetic strain distribution. The ranges of the physiological strains and related bone adaptive properties are reported in [11, 16]. There are upper (>3000 $\mu\varepsilon$) (< 50 $\mu\varepsilon$) that do not favour healthy bone growth. Strain ranges of adaptive bone growth are:

- Bone resorption (< 50 $\mu\varepsilon$),
- Remodelling (50-1500 $\mu\varepsilon$)
- Organized growth (1500-3000 $\mu\varepsilon$),
- Resorption (> 3000 $\mu\varepsilon$)

Biomimetic aspects are investigated by using the osteoconductive hybrid nano-composite coupled with a FEM modelling of the hybrid material swelling and deformation. The proposed CAD solid model of the new ceramic-polymeric modified implant is shown in Fig. 3.

Two biomechanical functions have been considered while designing the implant: *implant fixation and bone growth stimulus*. Portion of the Ti screw has been replaced by the hybrid nano-composite, that maintains the external thread continuity while internally, where the remaining Ti core is tapered trough the screw tip, shows a thickness varying from 0.5 to 0.8 mm. This thickening of the hydrophilic ceramic-polymeric hybrid nanocomposite produces, after swelling, a progressive volumetric increase across the tip. The scaffold should play two biomechanical functions: a structural one, as part of the fixture, and a bioactive one, as bone growth stimulus. Physiologically, the stretching of the periodontal ligament causes new bone apposition in the tooth socket. Since the elastic modulus of the swollen scaffold was comparable to that of the periodontal ligament, the Finite Element Analysis confirmed that the swelling of the nanocomposite could act as a biomechanical bone growth input.

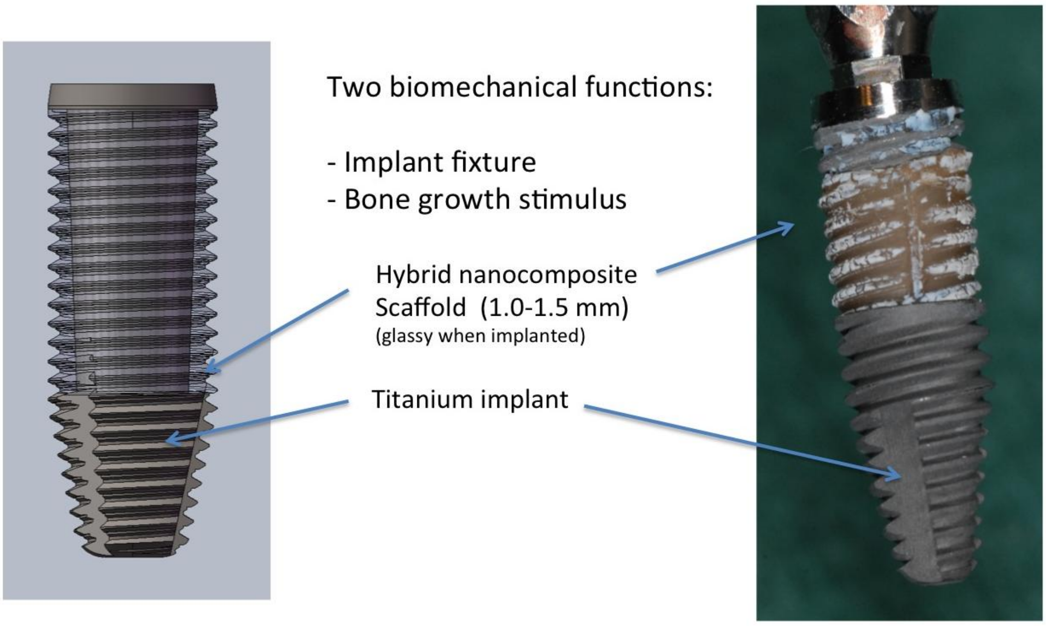


Figure 3. Thick elastic scaffold hybrid material mimicking periodontal ligament functions in the Biomimetic implant: CAD solid model and a prototype for use in “in vivo” tests of the new ceramo-polymeric modified Titanium implant

Fig. 4 shows the results of a Finite Element Analysis performed on the new implant simulating the ceramic-polymeric insert swelling in a physiological fluid: displacement up to 0.2 mm have been calculated (after 8 hrs. according to the 0.1 mm/hr swelling rate). This occurrence favours prosthetic device fixation and stabilization after implantation (*First biomechanical funct*ion).

Moreover, considering that remodelling is triggered not by principal stress but *strains* and that *dynamic loads* (not static load) on bone trigger remodelling, a positive growth stimulus is given by the presence of a deformable hybrid rubbery interface undergoing physiological (50-3000 $\mu\varepsilon$) compression and tension straining during bone healing and implant osseointegration (*Second biomechanical function*).

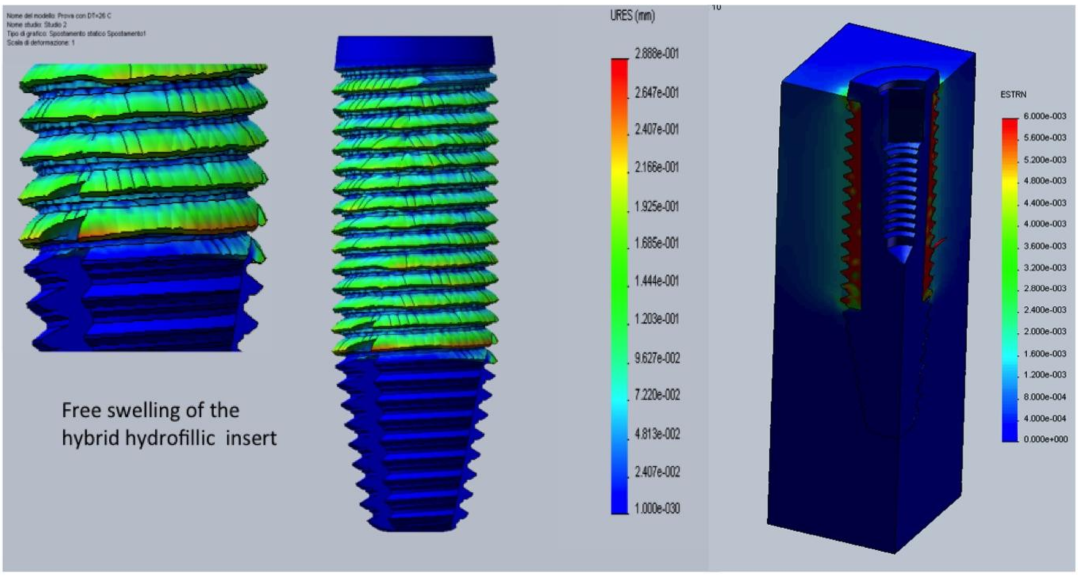


Figure 4. Displacements (URES) in mm and strains (ESTRN) of the hybrid ceramo-polymeric insert undergoing phisiological fluid free and constrained swelling after implantation.

Hybrid ceramic-polymeric insert swelling then stabilizes the implant in the bone and creates a biomechanically active interface for bone growth. Stresses on the bone have been modulated by scaffold swelling thickness choice for healthy bone growth. In vivo tests performed using this new modified oral implant confirmed the improved capability of such implants in promoting early osseointegration.

The present in vitro study aimed at evaluating the possibility of improving the primary stability of oral implants by means of three-dimensional scaffolds made up of an innovative hybrid ceramic-polymeric nanocomposite material.

In the test groups, the mean removal torque values progressively increased over time (diamond dots), ranging from 61.2 after 1 hour to 86.2 Ncm after 24 hours, showing how the swelling of the scaffolds improved implant primary stability. Conversely, in the control groups (square dots), the mean removal torque values ranged between 43.7 and 44.9 Ncm.

Fig. 5 compares the mean removal torque increases and sorption/swelling kinetics.

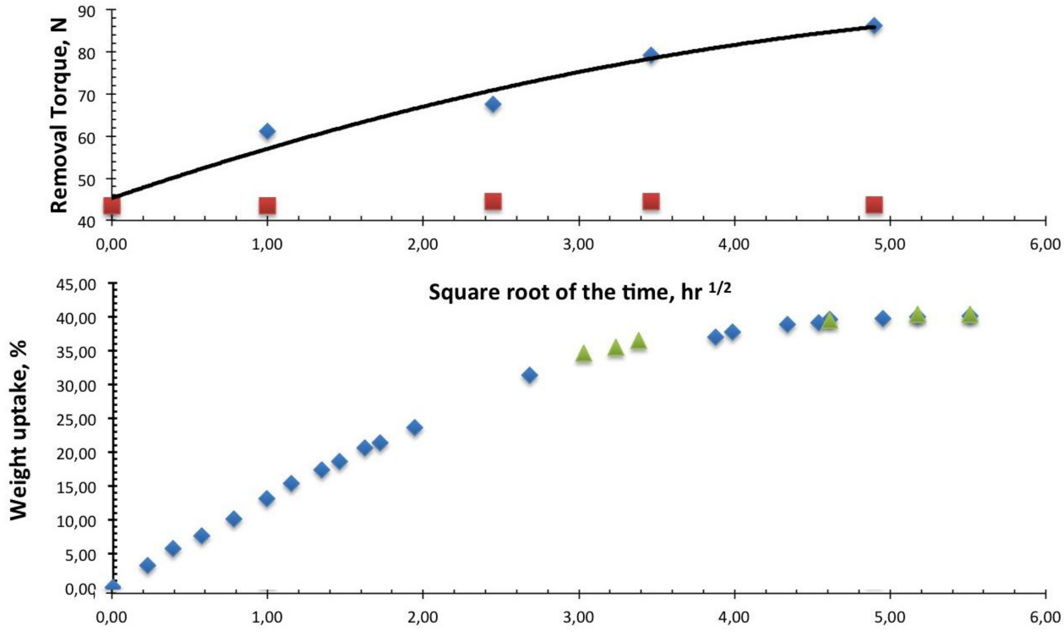


Figure 5. Comparison between the kinetics of after implantation removal torque increases and phisiological fluid uptakes in modified implants undergoing swelling.

The improvements of the removal torque over the time follow the same kinetic of the physiological solution uptakes experimentally determined in independent sorption tests.

'In vivo' Evaluation of Implants Bioactivity and Osteo-inductivity

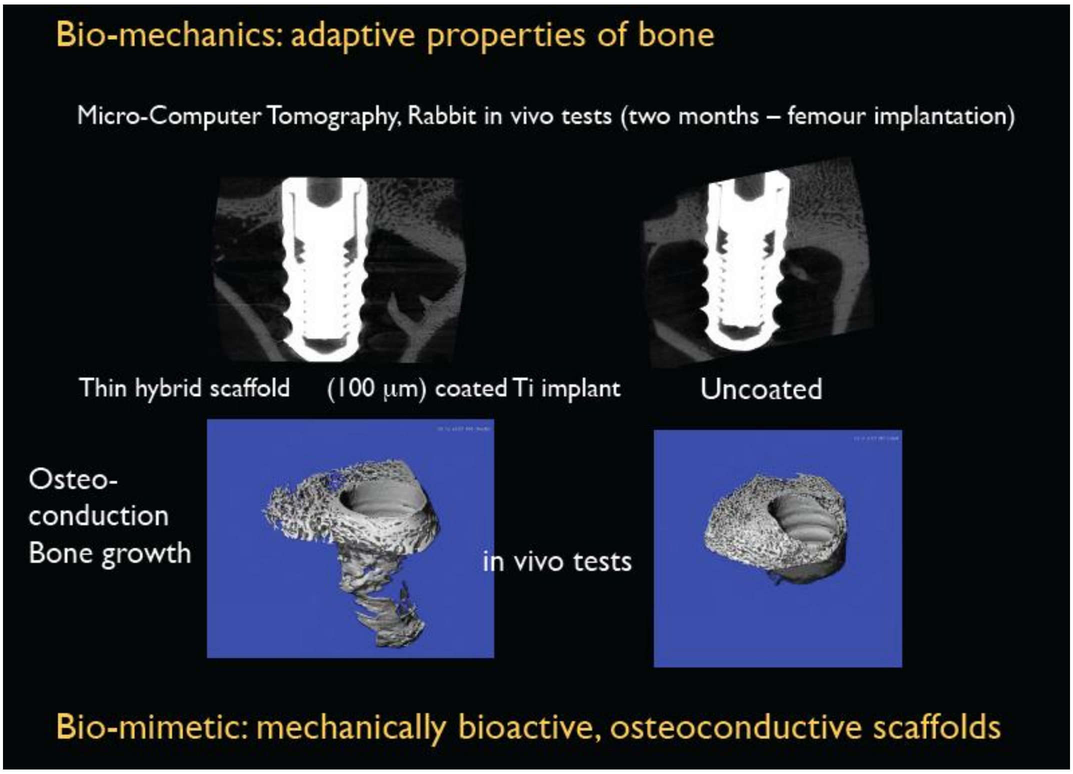
The results of the Micro CT investigation are reported in Fig. 6. The thin hybrid scaffold coated implants have shown two main relevant differences in osteo-inductivity and bioactivity compared to the unmodified implants:

- avoid bone resorption in the cortical bone surrounding the implant neck,
- improved osteo-induction in the medullar bone.

Uncoated Titanium implants have shown significant bone resorption at the cortical bone level.

This effect can be related to the improper biomechanical stimulation on the cortical bone outside the range of the 50-3000 με. To avoid this undesired effect due to the proper mechanical stimulation induced by the bioactive hybrid material scaffold interface.

Moreover, the bone growth on the implant surface (left implant in Fig. 6) is due to the osteo-inductivity of the tested hybrid scaffolding material.



Firgure 6. Biomechanicanics in adaptive morphology of the bone and osteoinduction in the hybrid scaffolding materials

Conclusions

A biomimetic/biomechanical approach has been pursued in designing new bioactive ceramic-polymeric hybrid material modified odontostomatological implant for biomechanical stimulation and potential improved scaffold mineralization and ossification.

A new nano-composite hybrid ceramic-polymeric Hydroxyl-Ethyl-Methacrylate polymer (pHEMA) filled with nanosilica particles (4-6% by volume) have been chosen as biomimetic material. This material swells (about 14% linearly) in presence of aqueous physiological solution (when in a biological aqueous environment) picking-up to 50-30% by weight of water (depending on nanosilica loading) turning from glassy and rigid to soft and rubbery. Mechanical behaviour of the proposed hybrid materials are comparable with those of bone when in the glassy state, and to those of the cartilage (ligaments) when rubbery after swelling.

The mechanical bio-mimicking properties of this highly osteoconductive [7] biomaterial have been utilized to develop a new bioactive odontostomatological implant. The new concept is driven by the consideration that a bioactive scaffolding interface between the implanted bone and the prosthetic device is generated when the material is able to stimulate the implant surrounding bone in the physiological strain range for healthy bone remodelling and organized growth (50-3000) [10-15].

The use of mechanically compatible hybrid hydrogels as scaffolding materials are expected to increase prosthesis adaptation mechanisms introducing active interfaces that improve implant biomimetic while reproducing cartilage and ligaments biomechanical functions. Adaptive properties of bone benefit of use of biomimetic (biomechanically compatible and bioactive) scaffold biomaterials coupled with new designed odontostomatological prostheses.

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