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Title: From wood to bone: multi-step process to convert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering

The remarkable features of natural bio-structures depend on their hierarchic structure which is an organized assembly of structural units at increasing size levels. It follows the primary importance of developing breakthrough processes to transform plant-derived hierarchical structures into biomedical devices for bone substitution and regeneration with smart anisotropic performances.

As featured in:

From wood to bone: multi-step process to convert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering

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Hydroxyapatite (HA) bone scaffolds characterized by highly organized hierarchical structures have been obtained by chemically transforming native woods through a sequence of thermal and hydrothermal processes. The whole chemical conversion has been carried out through five steps from native wood to porous hydroxyapatite: 1) pyrolysis of ligneous raw materials to produce carbon templates characterized by the natural complex anisotropic pore structure; 2) carburization process by vapour or liquid calcium permeation to yield calcium carbide; 3) oxidation process to transform calcium carbide into calcium oxide; 4) carbonation by hydrothermal process under CO₂ pressure for the further conversion into calcium carbonate; 5) phosphatization process through hydrothermal treatment to achieve the final hydroxyapatite phase. The five steps of the phase transformation process have been set up in order to achieve total phase conversion and purity maintaining the original native microstructure. An innovative biomimetic apatite hierarchically structured in parallel fastened hollow microtubules has been synthesized, structurally characterized and proposed as a new inorganic biomorphic scaffold providing a biomimetic nanostructure surface for fascinating bone engineering applications.

Introduction

In the last decade, there was an increasing demand for replacing traditional autograft and allograft bone substitutes with innovative biomaterials, able to replace, repair or even regenerate injured or diseased tissues. These new materials are required to perform repairing strategies with mechanically and biologically functional constructs able to support the wounded site and hasten the body’s natural repairing mechanism until new bone has filled the defect.¹

Human bone is a porous bio-hybrid composite, formed mainly from hydroxyapatite (70 wt%) and collagen (30 wt%). Bone structure exhibits gradients of both density and anisotropic properties: in fact bone, like the majority of bio-structures, is characterized by a complex hierarchy of structures. The anisotropic structural features provide stability, not only under compression and tension stresses, but also against bending and buckling.²,³

The peculiar structure of bone also allows an efficient distribution of the mechanical loads up to the smallest trabeculae, where the strain-sensing mechanism exhibited by the osteocyte cells can take place and activate the permanent bone remodelling process.⁴,⁵

During the last few decades, a variety of synthetic inorganic, polymeric and hybrid scaffolds has been developed in order to reach a good compromise in terms of mimicking the bone micro- and macro-porosity.⁶,⁷ Indeed, open-pore geometries with highly porous surfaces and microstructures allowing cell in-growth and reorganization and providing the necessary space for vascularization have been obtained.¹⁰,¹¹

On the other hand the optimal bio-physical response can be expressed only by a scaffold endowed with a structure exhibiting a high degree of hierarchy. In fact an ordered structure reflects not only the optimal mechanical features of the native scaffolds but, following the theory of the tensegrity model (for which the tissue organization depends on the spatial and mechanical stimuli exerted on cells by the scaffold), also the formation of well organized and performing remodelled bone.¹²,¹³

For these reasons the development of hierarchically organized bone scaffolds would represent a breakthrough in the knowledge in this field, surpassing the current solutions in the synthetic bone substitutes matter.

Regrettably, the current inorganic scaffold production processes do not allow the generation of a biomimetic organized hierarchical structure, due to the consistent limitations in the current chemical processing technology for biomaterials.¹⁴,¹⁵

In this view, Nature can be a source of inspiration for the development of hierarchically structured biomaterials.¹⁶,¹⁷ Nanotechnology has great potential in increasing the efficiency of biomimetic reconstructions by orders of magnitude. In fact, the nano-size of biological tissue building blocks is one of the bases of their self-organization ability and needs to be replaced by synthetic materials from the point of view of the synthesis of micro-structured architectures with controlled organization on multiple length scales.¹⁸,¹⁹

Following this approach, natural wood templates have been selected as a starting point: in fact, at the macroscopic level,

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wood can be considered as a cellular solid mainly composed of parallel hollow tubes, “the wood cells”. The cell wall is a fibre composite made of cellulose micro-fibrils embedded into a matrix of hemicellulose and lignin.\textsuperscript{20,21} The alternation of fibre bundles and channel-like porous areas makes wood an elective material to be used as a template in starting the preparation of a new bone substitute characterized by a biomimetic hierarchical structure. Wood cells can be roughly distinguished into tracheidal, parenchymal and libriform cells according to their transportation, storage and mechanical strengthening functions respectively.\textsuperscript{20} Thanks to the unique hierarchical architecture of the cellular micro-structure, wood exhibits a remarkable combination of high strength, stiffness and toughness at low density.\textsuperscript{21}

In the past, biomorphic ceramic structures derived from the conversion of wood have been obtained in a variety of compositions, through a sequence of infiltration reactions aimed at coating the inner surfaces of the wood tissue with oxide precursors, which gave rise to consolidated oxide ceramics (\(\text{Al}_2\text{O}_3\), \(\text{ZrO}_2\), \(\text{TiO}_2\), \(\text{MnO}\));\textsuperscript{22–26} alternatively, carbon templates obtained by pyrolysis of natural wood precursors have been infiltrated and directly transformed into carbide phases (\(\text{SiC}, \text{TiC}, \text{ZrC}\)).\textsuperscript{27–30} Particularly, \(\text{Si/\text{SiC}}\) wood-derived structures were optimized to be employed as bioinert bone scaffolds.\textsuperscript{31,32} Bio-mimesis of synthetic biomaterials can be carried out at different levels such as composition, structure, morphology, bulk and surface chemical–physical properties and reactivity. However, all the previous ceramic structures derived from the conversion of wood exhibit a bio-mimesis limited to the inner morphology and organized structure; their chemical composition and surface bio-activity are far from the biomimetic concept.

The aim of the present work is to obtain a new biomimetic hydroxyapatite bone scaffold having highly organized micro- and macro-porosity. For this aim a new series of complex chemical process has been investigated starting from natural wood and implying a multi-step procedure: pyrolysis of ligneous raw materials to produce carbon templates; vapour or liquid calcium infiltration to transform carbon into calcium carbide; oxidation process to yield calcium oxide; carbonation by hydrothermal autoclave treatments in controlled environment for further conversion to calcium carbonate and, finally, phosphatization through a hydrothermal process to synthesize biomimetic hydroxyapatite hierarchically organized scaffolds. Following these five successive chemical–physical transformations of a natural wood template, an innovative porous scaffold hierarchically structured in parallel fastened hollow microtubules has been synthesized and proposed as a new inorganic substitute for bone tissue replacement. Such synthesized structures maintain the 3D porous morphology of the starting native wood, thus allowing cell in-growth and reorganization and consequently providing the necessary space for vascularization.

**Results and discussion**

Scheme 1 illustrates the whole process transforming the starting wood into hydroxyapatite porous scaffold. The criteria for the natural wood selection as starting materials were based on the specifications of spongy bone regions characterized by biomechanical properties able to fit the demand of anisotropic stresses.

**Pyrolysis**

The pyrolysis of the wood specimens provoked the decomposition of the organic part, namely the polysaccharides (cellulose, hemicelluloses) and the lignin, which represents the wood main weight fraction. Pyrolysis was carried out at 1000 °C using a slow
heating rate (1 °C/min) to prevent cracking of the carbon template. H₂O, CO and CO₂ gases were released during the pyrolysis process and, in spite of the huge shrinkage and the large weight loss recorded during the process, the slow heating rate assures the preservation of the typical anisotropy and organized microstructure of the native wood morphology (Fig. 1c, d).

Carburization

Carbon templates obtained by pyrolysis were transformed into calcium carbide (CaC₂) by a carburization process which was carried out following two different methods: a) liquid phase and b) vapour phase infiltration.

Both the infiltration processes require suitable heating treatments, where the calcium firstly reacts with the carbon surface and then diffuses into the core of the newly formed structure, proceeding its reaction with the un-reacted inner carbon. Vapour phase infiltration was performed at higher temperatures, above the boiling point of calcium (1484 °C). It was found that vapour infiltration was more effective than liquid infiltration in retaining the initial structure of the organic template mainly at the micrometer level. In fact, in this case the reaction with the carbon templates occurred at a molecular level. The chemical reaction involved is:

\[
2C + Ca \rightarrow CaC_2 (\Delta H = -15.37 \text{ Kcal}) \tag{1}
\]

The obtained CaC₂ final structures exhibit an improved toughness compared to the carbon template, but could be easily degraded by moisture following the reaction:

\[
CaC_2 + 2H_2O = Ca(OH)_2 + C_2H_2 (\Delta H = -28.02 \text{ Kcal}) \tag{2}
\]

so that, once extracted from the furnace, the CaC₂ pieces must be preserved in an inert gas environment or in vacuum conditions. On the other hand, this phenomenon provides a quick test for the successfulness of the carburization process: calcium carbide strongly reacts with water, forming white calcium hydroxide and acetylene gas, so the absence of any residual black un-reacted carbon indicates the complete conversion of carbon in calcium carbide.

After the rapid increase of the temperature to 800 °C and a slower heating to 1100 °C, the following step, set up in the temperature range 1550–1850 °C (higher than the calcium boiling point, 1484 °C), was carried out at a slower heating rate (1 °C/min) to allow controlled evaporation of calcium. In fact the Ca source is often surrounded by a thin layer of calcium oxide which the evaporating core has to slowly overcome avoiding a violent reaction detrimental for careful control of the carburization process. In the case of rattan, the first step of the thermal cycle (25–800 °C) was slowed (from 10 to 5 °C/min) to avoid the crumbling of the specimens.

The dwell at the final temperature was set up both to allow the complete reaction of carbon with calcium and to eliminate the excess of calcium by evaporation. The residual un-reacted calcium content depends on the total porosity, the pore channel diameter distribution and the calcium amount deviation from the stoichiometric value, so that the carburization process should be set up accordingly.

All the processes were carried out under argon atmosphere, preventing the oxidation of the calcium source, the ignition of pyrolysed wood and the hydration of the formed calcium carbide.

During vapour phase infiltration of pine wood derived carbon templates, the thermal cycle was set up to achieve T = 1650 °C. In fact, below this temperature, the reaction is not complete and part of the starting carbon could remain un-reacted, while at higher temperature a partial deformation of the calcium carbide network could be detected.

After calcium vapour infiltration, the original cellular structured network of the pyrolysed wood is preserved, even if the total pore volume in the sample decreases, due to the volume expansion of the struts induced by the conversion into CaC₂. The morphology of the cellular framework of pine and rattan wood after the transformation phase from carbon into calcium carbide is shown in Fig. 2a and b, respectively. The conversion of carbon into CaC₂ induces an appreciable volume increase of the solid and the formation of micro-pores can be supposed, where the gas can get in. These features support possible reaction mechanisms active during the vapour infiltration process: the infiltration of the gaseous calcium into the carbon template; the heterogeneous gas/solid reaction between calcium gas and carbon to form CaC₂ at the pore surface and, finally, the diffusion of calcium gas through the newly formed CaC₂ scale.

The strut thickness between pores is affected by the Ca/C molar ratio used in the carburization reaction and increases with the Ca/C molar ratio. The best result in terms of required strut thickness and pore diameter was obtained using a Ca/C molar ratio = 1. In fact, when this reagent ratio is used in the carburization reaction, the strut thickness appears in the range 4–6 μm inducing consequently the smallest reduction of large pore voids (Fig. 3b).

The capability of Ca vapour to penetrate into the porous carbon templates is one of the most important criteria for
massive in-pore reaction and the extensive homogeneous formation of calcium carbide through the whole structure of the carbonized wood template. In fact, the XRD and SEM-EDS analyses reveal the nucleation and growth of the CaC₂ phase leading to the formation of a continuous homogeneous CaC₂ layer without the presence of secondary phases.

As concerns rattan wood, the volume shrinkage was much higher than in pine wood, being about 33%, but also in this case the anisotropic morphology was perfectly retained (see Fig. 2b).

Oxidation

Porous calcium carbide template samples derived from pine wood were transformed into calcium oxide templates by the oxidation process described as follows:

\[ 2\text{CaC}_2 + 5\text{O}_2 \rightarrow 2\text{CaO} + 4\text{CO}_2 (\Delta H = -651.05 \text{ Kcal}) \quad (3) \]

The process was carried out using an optimized heat treatment, in order to ensure the complete conversion into CaO and to preserve the morphology of the native wood. In order to set up the most suitable thermal cycle, X-ray diffraction analysis (XRD) has been utilized to identify the crystalline phases obtained by thermal treatment of the CaC₂ template as a function of the used temperature. XRD spectra were collected on a CaC₂ template thermally treated at different final temperatures in the range 300–900 °C in air (Fig. 4a). The results of the XRD analysis revealed the presence of Ca(OH)₂ in the CaC₂ template samples which begins to decay between 300 and 400 °C, while CaCO₃ gradually becomes the dominating crystalline phase according to the reaction:

\[ 2\text{CaC}_2 + 5\text{O}_2 = 2\text{CaCO}_3 + 2\text{CO}_2 (\Delta H = -736.46 \text{ Kcal}) \quad (4) \]

Upon increasing the temperature, the presence of CaC₂ and CaCO₃ continuously decreases to completely disappear at 800 °C.

The presence of CaO can be detected starting from 400 °C and increased due to the Ca(OH)₂ decomposition which takes place at about 450–500 °C according to the formula:

\[ \text{Ca(OH)}_2 = \text{CaO} + \text{H}_2\text{O} (\Delta H = 18.98 \text{ Kcal}) \quad (5) \]

At T = 800 °C the presence of CaO abruptly rises, due to CaCO₃ decomposition:

\[ \text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2 (\Delta H = 40.06 \text{ Kcal}) \quad (6) \]

so that at T = 1000 °C this is the only crystalline phase present. Furthermore, the transition from the tetragonal form of calcium carbide (CaC₂(I), ICDD card no. 51-1167) to the monoclinic one (CaC₂(II), ICDD card no. 1-962) at about 500 °C can be inferred even if the involved XRD reflections appear very weak.

A schematic drawing of the crystalline phases volume percentage formed as a function of the heat treatment temperature is reported in Fig. 4b.

Carbonation

Calcium carbonate pieces preserving the typical microstructure of the native wood were obtained from calcium oxide pieces previously prepared following the reaction:

\[ \text{CaO} + \text{CO}_2 \rightarrow \text{CaCO}_3 (\Delta H = -41.99 \text{ Kcal}) \quad (9) \]
by two different processes: either fluxing CO2 gas inside a furnace chamber or by employing compressed CO2 into a hydrothermal reactor.

The transformation reaction was found to proceed through two rate controlling regimes: the initial stage concerns the heterogeneous gas–solid CO2–CaO interfacial reaction; then, when a compact layer of CaCO3 is formed on the surface of CaO, the rate limiting step is the diffusion of CO2 through the CaCO3 layer.

The full conversion of CaO into CaCO3 was obtained by high pressure treatments in a hydrothermal synthesis reactor. The employment of high pressure values allows the penetration of CO2 across the forming CaCO3 scale, up to the core of the CaO structure, and increases the rate of carbonation.

The conversion extent of CaO templates into porous CaCO3 was not directly related to the employed temperature, but it depends on the CO2 pressure used to allow the full penetration to the inner part of the CaO template.

The XRD patterns of CaCO3 templates obtained according to the two different working conditions show the presence of CaCO3 as the main crystalline phase, even if some traces of Ca(OH)2 are detected. Conversely, the carbonation process carried out with a hydrothermal reactor leads to the complete carbonation of CaO (Fig. 5).

The SEM images reported in Fig. 6a and b show the microstructures of the CaO templates before and after the carbonation treatment, respectively, revealing that the typical hierarchically organized anisotropic morphology of the native wood has been preserved after the carbonation process.

**Phosphatization**

The transformation of calcium carbonate templates into porous hydroxyapatite ceramics was performed using KH2PO4 as a phosphate source, following the reaction:

$$10\text{CaCO}_3 + 6\text{KH}_2\text{PO}_4 + 2\text{H}_2\text{O} = \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{KHCO}_3 + 4\text{H}_2\text{CO}_3 \quad (\Delta H = 13.149 \text{ Kcal}) \quad (10)$$

The XRD pattern of the porous template obtained by hydrothermal reaction in aqueous phosphate solution only exhibits the diffraction maxima belonging to hydroxyapatite (HA) and confirms the complete transformation of calcium carbonate templates into pure HA as the final product (Fig. 7). Due to the selected hydrothermal pH condition (pH ≈ 7.4) and calcium concentration, the most thermodynamically stable phase is HA and in addition calcium ions cannot re-precipitate as calcium carbonate, according to the solubility product value of HA ($K_{ps} \approx 10^{-32}$, in the case of carbonated hydroxyapatite (CHA): $K_{ps} \approx 10^{-36}$), which is much lower than that of calcite ($K_{ps} = 7 \times 10^{-8}$).

Moreover, besides the typical HA bands, FTIR analysis showed a small band at 880 cm$^{-1}$ and a doublet centred at 1440–1550 cm$^{-1}$, suggesting the presence of carbonate groups substituted in the HA lattice (Fig. 8), as also observed in natural

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**Fig. 5** XRD pattern of a pine wood-derived calcium carbonate piece obtained by the hydrothermal conversion process.

**Fig. 6** SEM images of pine wood-derived calcium oxide a) and after hydrothermal conversion into calcium carbonate preserving the natural wood microstructure b).

**Fig. 7** XRD pattern of wood-derived hydroxyapatite.

**Fig. 8** FTIR spectrum of wood-derived hydroxyapatite.
HA of human bone. Thus, the whole transformation process enabled the formation of a material characterized by increased bioactivity, compared to stoichiometric HA, potentially able to accelerate the process of bone remodelling. Well crystallized hydroxyapatite obtained as final products by pyrolysis, carburization, oxidation, carbonation and phosphatization of selected wood templates maintained a highly anisotropic microstructure. The SEM images in Fig. 9 demonstrate that the structure anisotropy typical of the native woods was preserved on the macro-scale, exhibiting in the case of rattan pore sizes in the range 100–300 μm. Detailed images of biomorphic HA derived from pine wood, reported in Fig. 10, reveal an ordered fastening of parallel micro-tubes 100–150 μm long and 15–30 μm wide with a hollow core of about 10–25 μm in diameter, organized like the cell morphology of the natural wood used as the starting template for its synthesis. The porosity evaluation displayed in Fig. 11 confirms the presence of nano/micro-porosity ranging from 0.01 μm to over 100 μm. A soaking test was also performed using red Hanks’ solution: the rapid colouring of the scaffold, thanks to fluid imbibition, confirmed the high level of interconnectivity due to the micro- and nano-porosity. In the experimented conditions a chemical exchange reaction can be supposed by which the porous calcite structure is preserved as final HA; on the other hand, the higher magnification (see insert in Fig. 10b) of the newly formed HA surface morphology shows typical needle-like nuclei grown on the surface, proving the concomitant occurrence of a dissolution/precipitation process at sub-micron level, in agreement with hypotheses previously reported.44,45 This surface nanostructured morphology of the unidirectional fastened hollow HA microtubules allows biological systems like cells to utilize biomorphic scaffolds on the micrometer level which are also biomimetic for composition and structure on the nanometer scale. Preliminary mechanical evaluation showed that the compression strength of the scaffold derived from pine wood, measured in the direction parallel to the channels, ranged between 2.5 and 4 MPa, while in the direction perpendicular the value it was lowered to 0.5–1 MPa. The compression strength measured on isotropic bone scaffold (Engipore®) was 3.5 MPa in all directions, and completely deproteinated bone is usually too brittle to be measured.46

**Experimental**

**Raw materials**

The criteria for the natural wood selection were based on the isolation of regions resembling spongy bone and exhibiting fairly mechanical properties. Two different wood specimens were selected considering the total porosity and the pore size distribution: pine wood and rattan (Manau). The starting samples were obtained by cutting the native woods in parallelepiped pieces of about 1 cm in lateral dimension.

**Pyrolysis**

The pyrolysis process was performed in mild argon flux on parallelepiped specimens of wood in its native form. The temperature was raised from room temperature at 1 °C/min to 1000 °C and, after a dwell of 1 hour, decreased at 2 °C/min to ambient temperature.

**Transformation of pyrolysed wood into calcium carbide**

The experiment was set up as follows: two holes were created on the lateral walls of a cylindrical graphite crucible using a drill of 1 mm diameter, similarly a piece of pyrolysed wood was also drilled right through. A supporting pencil lead in graphite (thickness: 0.9 mm) was used, passing through the wood and the crucible; the crucible so prepared was overturned and positioned upon a second crucible, of similar size, filled with metallic calcium granules (Sigma-Aldrich). A reaction chamber was so

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![Fig. 9](image1.png) **Fig. 9** SEM images of wood-derived HA: a) pine wood; b) rattan.

![Fig. 10](image2.png) **Fig. 10** Detailed SEM images of pine wood-derived hydroxyapatite: a) microstructure of wood-derived parallel fastened hydroxyapatite micro-tubes; b) typical needle-like HA nuclei grown on the micro-tube surface.

![Fig. 11](image3.png) **Fig. 11** Pore size distribution of rattan-derived HA.
The infrared spectra were measured from 4000 to 400 cm\(^{-1}\) with 2 cm\(^{-1}\) resolution using a Nicolet 380 spectrometer. Other settings include an 8 mm aperture, 32 scans, velocity of 10 kHz, DLATGS detector, and a 3-term Blackman-Harris apodization function. KBr pellets were obtained under vacuum, using 1 mg of the powdered samples carefully mixed with 200 mg of infrared grade KBr.

**FTIR analysis**

The transformation of CaC\(_2\) specimens into calcium oxide porous ceramics was carried out by heating in air at 10 °C/min to 500 °C, then a slower heating (2 °C/min) was applied to 900 °C for 1 hour.

**Hydrothermal carbonation of CaO templates**

The phase transformation of wood derived CaO templates in calcium carbonate was carried out into a hydrothermal synthesis reactor (Parr Stirred “Mini” reactor model 4652) with a 500 cm\(^3\) moveable vessel constructed with “alloy C-276” at a temperature of 400 °C for 24 hours and filled with CO\(_2\) at a pressure of 2.2 MPa.

**Hydrothermal phosphatization of CaCO\(_3\) templates**

The wood derived CaCO\(_3\) templates were soaked in a KH\(_2\)PO\(_4\) aqueous solution (CO\(_3\)/PO\(_4\) molar ratio 5/3) and treated in a hydrothermal synthesis reactor, at T = 200 °C, P = 1.2 MPa, pH = 7.4 for 24 hours. The ΔH of each chemical reaction was calculated using HSC Chemistry® 6.0 software that is provided with extensive thermo-chemical database and flow sheet simulation.

**X-Ray diffraction analysis (XRD)**

XRD data were collected using a PANalytical 0/0 diffractometer (CuK\(_\alpha\) radiation, 40 kV and 40 mA), equipped with a real-time multiple strip (RTMS) detector. Data were collected in continuous mode, with a Ni filter on the primary beam, 1/4° fixed divergence slits, 0.0023° soller slits on both the incident and secondary beams, 1/4° fixed anti-scatter slit, and fixed 5 mm RTMS slit. An integrated step scan of the RTMS detector of 0.0167°/20 was used with a counting statistics of 30 s/step.

The phase composition and the microstructure of the raw, intermediate and final crystalline products in the whole transformation process were quantitatively estimated through the Reference Intensity Ratio (RIR) method by employing peak intensity ratios (reference: corundum) calculated by the software Powder Cell v. 2.4 (Kraus W, Nolze G, Federal Institute for Materials Research and Testing, Berlin, Germany). XRD spectra of wood-derived CaC\(_2\) pieces were also collected in high temperature attachment in the range 300–900 °C in air (CuK\(_\alpha\), Geigergel IV, Rigaku, Tokyo), to thoroughly investigate the oxidation step. The examined samples were 16 × 12 × 1.5 mm\(^3\) plates firmly mounted on a platinum sample holder.

**Porosity evaluation**

Mercury porosimetry was used to evaluate the pore size distribution (<100 μm) using a Porosimeter Unit 120 (Carlo-Erba, Italy).

**Mechanical properties evaluation**

The compressive strengths of the new scaffold both parallel and perpendicular to the channel direction were evaluated by using a universal screwtype testing machine (Instron 6025), with a crosshead speed of 0.5 mm/min. A population of five samples for each investigated material was employed for the evaluation of mechanical properties.

**Conclusions**

In summary, the novel multi-step process described above was shown to be able to transform native vegetable structures into apatitic inorganic biomaterial mimicking the original 3D morphology and hierarchical architecture of the original biological template with unidirectional oriented pore structures on the micrometer scale. The high versatility of this multi-step process allows facile chemical control of the product and thus the possibility to modulate and improve the bioactivity of the final highly porous inorganic materials. The hierarchical architecture of the wood cellular microstructure and the hydroxyapatite constituting phase allow this new biomimetic material to be considered as an innovative charming inorganic scaffold for bone regeneration and engineering. The intrinsic worth of such a scaffold is its multi-level organized morphology characterized by tubular structures, as needed by vascular remodelling, macropores, allowing settling and proliferation of bone cells, and the fascicular matrix able to satisfy the biomechanical requirements.

Moreover, the feasibility of applying this novel technique to the innumerable different organic structures existing in Nature opens the way to a wide number of applications, for which...
hierarchically organized structures, not achievable by conventional manufacturing techniques like filtering and high mechanical impact devices, are required.

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