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Critical analysis on dynamic-mechanical performance of spongy bone: the effect of acrylic cement

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Abstract

Introduction

Over the past years, in order to anchor a prosthetic component to bone, different cements based on self-polymerizing poly (methyl methacrylate) (PMMA) have been widely used as commercial synthetic biomaterials. The aim of this study was to evaluate the influence of a PMMA-based bone cement (Palamed® G40) on the viscoelastic behaviour of spongy bone from proximal human tibial epiphyses, and the assessment of the dynamic-mechanical properties of the natural tissue.

Materials and methods

The effect of acrylic bone cement on the dynamic-mechanical performance of spongy bone was assessed by means of dynamic three-point bending tests. All the tests were performed in physiological solution at 37±0.5°C, scanning the frequency from 0.01 to 30 Hz. Samples from proximal human tibial epiphyses were cut along the medial-lateral and anterior-posterior directions considering different regions of the subchondral tibial plate. The dynamic-mechanical properties of specimens infiltrated the PMMA-based bone cement were evaluated in the frequency range investigated.

Results

The results have highlighted that the viscoelastic properties of spongy bone vary with direction and region, and the PMMA-based bone cement increases the storage modulus (E') of spongy bone of about 100%. However, for bone-cement system, the values of loss factor (tan d) are close to those of the natural structure. Moreover, micro-computed tomography (µCT) has allowed to study the architecture of bone and its interface with the cement.

Conclusion

The present study has evidenced that bone infiltration allows to enhance the mechanical performances of spongy bone. Furthermore, taking into account the analysis of the viscoelastic properties of the natural structure, future trends will be focused on the possibility to design a prosthetic implant, which emulates the biomechanical behaviour of the natural tissues, or a suitable cement, which improves the mechanical properties of spongy bone.

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Introduction

As for any total joint replacement, the primary goal for a total knee arthroplasty (TKA) is to restore normal joint function as long as possible. Therefore, the prosthetic joint must be able to provide the motions and to transmit functional loads that occur in a normal knee and to ensure a long life. In other words, it needs to avoid disruption of the interface between implant and bone and also to minimize articulating surface damage. In the last 25 years, considerable progress has been made and total knee prosthesis has proven to be an efficacious procedure to relieve pain due to arthritis and to restore the stability and the mobility of a damaged knee[1]. The knee is the largest and most complex human joints, whose purpose is to accept and transfer loads among the femur, tibia and patella, to dissipate loads generated at the end of the long mechanical lever arms of the femur and tibia[2]. The distal femur and the proximal tibia consist of cancellous bone and an outer cortical shell. In the distal femur, the load is transmitted from the dense subchondral bone to the cortical bone of the diaphysis over a short metaphyseal region. In the tibia, the load is gradually transmitted from the subchondral cancellous bone to the cortical cone of the diaphysis.

Therefore, the structural stiffness of cancellous bone gradually decreases in the proximal-distal direction, whereas the structural stiffness and thickness of the outer shell increase in the same direction[3].

Bone is a dynamic, living composite tissue and its extracellular matrix mainly consists of hydroxyapatite crystals (the mineral phase), type I collagen (90% of the organic phase) and water[4,5]. The high stiffness of bone is due to the mineral phase, while its viscoelastic behaviour is mainly related to the collagen macromolecular arrangement. On the other hand, water which is bonded on crystal surfaces for ion exchange stabilizes the collagen triple helix by realizing hydrogen bonds and it strongly affects viscoelastic properties[5,6,7,8,9,10,11].

Differently from cortical bone, spongy bone (also called trabecular or cancellous bone) is like a network of small needle-like pieces of bone that form an apparently irregular latticework called trabeculae and the porosity is filled with marrow[12].

The viscoelastic properties of bone result important in the case of fractures, which are generally related to dynamic loads and high-energy impacts such as a fall in a high-speed event[4].

Research focused on the evaluation of the mechanical properties of mineralised connective tissues (hard tissues) has been extremely active

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especially over the last half a century, it is still a basic research field motivated by at least three considerations: the first is to achieve a greater knowledge of the relationship between tissue structure and mechanical properties in order to develop diagnostic tools; the second is the need to improve the design of prosthetic devices used as hard tissue substitutes; the third is to improve the relationship between prosthetic joints and cortical or trabecular bone. The reliability and the significance of mechanical measurements of hard tissues are mainly dependent on anisotropy, time, sex, age, organ, site and adaptation in relation to the experienced loads, diseases or traumas.

The implant of a total joint prosthesis may induce altered stress distribution pattern, thus according to "Wolff's Law" bone adjusts its density and structure to the current mechanical demands. Many studies using finite element analysis demonstrated altered mechanical loading in the bones after implantation of a total joint prosthesis[13]. After uncemented TKA, a significant and progressive decrease of bone density below the tibial component, reaching 22% at 3 years follow-up, has been reported. In rheumatoid arthritis, quantitative measures of bone remodelling of the proximal tibia following TKA with a cemented non-metal backed tibial component found an average decrease in bone density of 32% after 2 years[14].

There is a significant diminishing of quality of bone beneath the tibial component after knee arthroplasty that is greater than would be expected with normal aging and it is well known that the failure of total knee arthroplasty may be related to changes in the quality of bone.

Fixation of total knee components with bone cement is considered the current "gold standard". Many studies have reported excellent long term results with cemented total arthroplasty and loosening of cemented components is a rare complication in most large clinical series[15,16]. Cements based on self-polymerizing poly(methyl methacrylate) (PMMA) are the most used commercial synthetic biomaterials for anchoring a prosthetic component to bone.

They consist of a solid powder phase made of PMMA and/or copolymers and a liquid monomer phase. Additionally, the powder contains dibenzoyl peroxide (BPO) as initiator for radical polymerization, a radiopaque medium and sometimes an antibiotic. The main components of the liquid phase are MMA and, in some bone cements, other esters of acrylic acid or methacrylic acid, one or more amines (i.e. activators/co-initiators for the formation of radicals), a stabilizer and, possibly, a colorant[17,18].

Thus, in order to optimize the developing of prosthetic implants and new diagnostic techniques, the knowledge of the structure-mechanical property-function relationships is crucial.

Mechanical properties of spongy bone have been evaluated according to different techniques.

The ultrasonic approach is often preferred since the simplicity of the specimen geometry and also because it easily allows to distinguish the mechanical anisotropy in the elastic properties of bone[19]. However, this method uses high frequencies, thus it is unable to measure viscoelastic properties related to normal dynamic condition of bone. The combination between shear and bending waves represents a further problem which leads to erroneous measurement and interpretation of moduli, though[20].

The nanoindentation technique is another interesting method to locally evaluate the mechanical properties of soft and hard tissues, using an atomic force microscope or a nanoindenter. The main drawback is that the reduced modulus (i.e. a combined modulus) obtained from nano-indentation measurements is related to the Young's moduli of tip and sample, and to the their Poisson's ratios. The tip properties are usually known, thus the Young's modulus of a material can be evaluated from the reduced modulus if the Poisson's ratio of the sample material is known. Consequently, when the Poisson's ratio of the sample is not known, a plane strain modulus is usually reported[21].

In addition, several nanoindentation methods have been also exploring to assess the viscoelastic properties of materials, and many nanoindenters present a dynamic testing option, where a sinusoidal excitation can be applied to evaluate storage and loss moduli as a function of frequency. In this context, the viscoelastic properties of different tissues, including bone, demineralized dentin, arteries and skin, have been investigated[22,23,24,25,26,27].

For example, a new technique using a combination of an atomic force microscope and a force-displacement transducer has been used to simultaneously investigate the surface topography and to map the mechanical properties of hard tissues such as human teeth.

In particular, this new modulus mapping method has allowed the evaluation of both storage and loss moduli for small areas of calcified tissues with high spatial resolution, providing the first quantitative determinations about the variations of the dynamic moduli at the dentin–enamel junction and peritubular–intertubular dentin junction[28]. However, many problems could arise from the plastic deformation of the area that may drastically alter the storage and loss moduli. Therefore, the classical mechanical approach seems to be an effective method to investigate the viscoelastic properties of hard tissues such as spongy bone.

The aim of this study was to evaluate the dynamic-mechanical properties of spongy bone from proximal human tibial epiphyses, and the effect of a PMMA-based bone cement on the viscoelastic behaviour of this tissue.

Materials and methods

The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed.

Samples 3.7 mm thick, 14 mm wide and 45 mm long from six proximal human tibial epiphyses (62±10 years) were cut along the medial-lateral and

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anterior-posterior directions from different regions of subchondral tibial plate using a water-cooled low-speed diamond saw (Figure 1a, Figure 1b and Figure 1c).

Specimens obtained from the same region and orientation were divided in two groups, group A and group B. Group B specimens were infiltrated with Palamed® G40 (Biomet-Merck, Germany) bone cement which contains gentamicin sulphate.

The cement was hand mixed for 120 s hence applied on the upper and lower surfaces of each specimen previously washed and hence dried with gauze, just as occurs during surgery. A constant pressure was applied during the cement setting.

Dynamic-mechanical tests were then carried out on group A and group B specimens.

The dynamic-mechanical tests in bending were performed in physiological solution at 37 ± 0.5°C by using an EnduraTEC ELF 3200 Series Bose. The three-point bending configuration with a support span of 42 mm was selected (Figure 1d). In order to evaluate storage modulus (E'), viscous or loss modulus (E'') and loss factor (tan δ), a sinusoidal strain characterized by a dynamic amplitude of 0.5 % was imposed. The frequency was scanned from 0.01 to 30 Hz.

The storage modulus and the viscous modulus are related to the energy stored in the material and to the energy dissipated during the loading process, respectively.

Stress and strain amplitudes (σ0 and ε0, respectively), storage modulus (E'), viscous (or loss) modulus (E'') and loss factor (tan δ) were evaluated according to the ASTM D 790 standard:

(1)

$$\sigma_0 = \frac{3F_0L}{2bd^2} \qquad E' = \frac{\sigma_0}{\varepsilon_0}\cos\delta \qquad \tan\delta = \frac{E''}{E'}$$

$$\varepsilon_0 = \frac{6D_0d}{L^2} \qquad E'' = \frac{\sigma_0}{\varepsilon_0}\sin\delta$$

where D0 is the applied middle span deflection amplitude, P0 is the monitored load amplitude, L is the span between supports, b and d are the sample width and depth, respectively. In order to investigate the architecture of bone and its interface with the cement, a micro-computed tomography (μCT) was also performed at a resolution of 5.8 μm through a SkyScan 1072 (Aartselaar, Belgium) system using a rotational step was 0.9° over an angle of 180°.

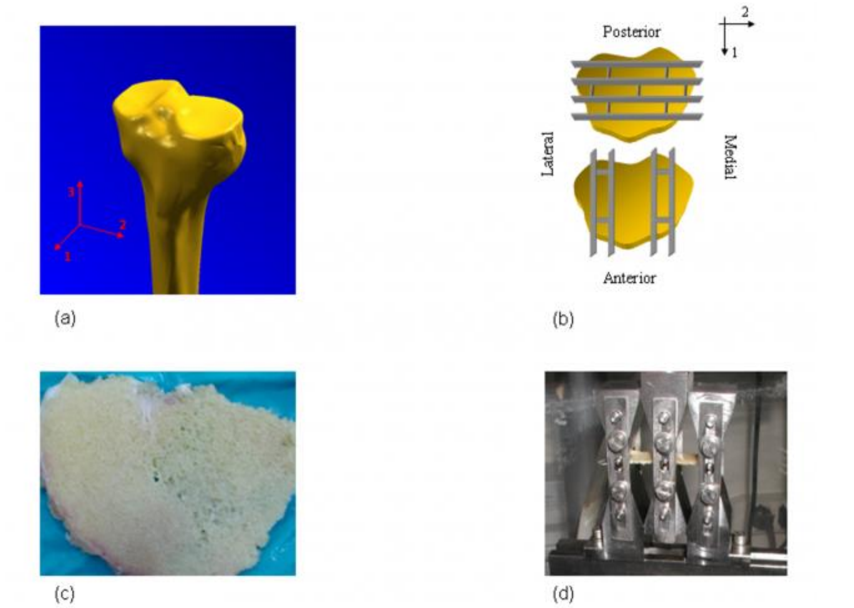

Figure 1: (a) Schematic representation of human tibial epiphysis; (b) Anatomic region and directions; (c) An image of human tibial section from the epiphysis; (d) Mechanical testing set-up.

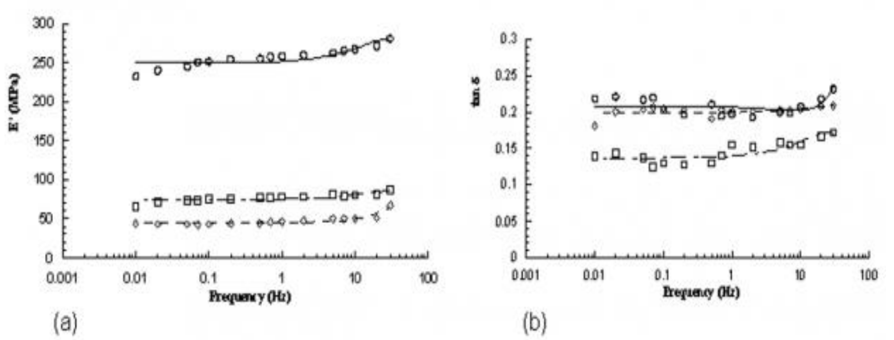

Figure 2: (a) Storage modulus as function of frequency for group A specimens cut along the medial-lateral direction from anterior zone (◊), posterior zone (□) and central zone of the medial aspect (○); (b) Loss factor as function of frequency for group A specimens cut along the medial-lateral direction from anterior zone (◊), posterior zone (□) and central zone of the medial aspect (○).

Results

Dynamic-mechanical measurements performed on spongy bone specimens have shown a storage modulus E' which increases with frequency for specimens provided from all the anatomic regions, indicating that the structure becomes stiffer as the strain rate increases (Figure 2a). In particular, specimens from the central zone of the medial aspect show that E' varies from 230.6 to 270.0 MPa (Figure 2a), whilst samples from the other positions have shown smaller values ranging from 42.8 to 66.7 MPa and from 65.1 to 87.0 MPa (Figure 2a) for the anterior zone and posterior zone, respectively. The great difference in the storage moduli can be due to a different degree of porosity and to a different water content. Also, the viscous modulus generally increases with frequency. E'' values range from 50.3 to 62.4 MPa for specimens from the central zone of the medial aspect, which are higher than

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the values obtained for the anterior and posterior positions. These values span from 7.9 to 11.3 MPa and from 9.0 to 13.3 MPa, respectively.

Interestingly, the trends and values of loss factor (tan δ) seems to be independent from the region. Similar values are found especially for specimens from central and anterior zones; tan δ values of specimens from the posterior position result slightly smaller than those from the other two anatomic sites (Figure 2b). In every region, tan δ values are smaller than 0.3 (δ<45°), suggesting that spongy bone dynamic-mechanical behaviour in flexure is predominantly elastic since the viscous component (E'') results smaller than the elastic one (E').

Similar to medial-lateral direction, differences due to the position have also been found for the anterior-posterior direction, in which specimens from intercondilar zone have shown E' values (from 36.7 to 43.7 MPa) smaller than those from medial zone (from 245.7 to 271.8 MPa) (Figure 3a).

Dynamic-mechanical analysis on group B specimens also suggests that even if bone cement increases the storage modulus (E') of spongy bone of about 100% for all the specimens from the several regions (Figure 3a), tan δ values are close to those of the natural structure, though (Figure 3b).

Furthermore, mCT analysis (Figure 4) clearly shows that the bond between cement and spongy bone is mainly micro-mechanical. Accordingly, cement sprouts reaches depth higher than 0.5 mm, however a lack of interface between the cement and bone is evident already at a depth of 0.2 mm.

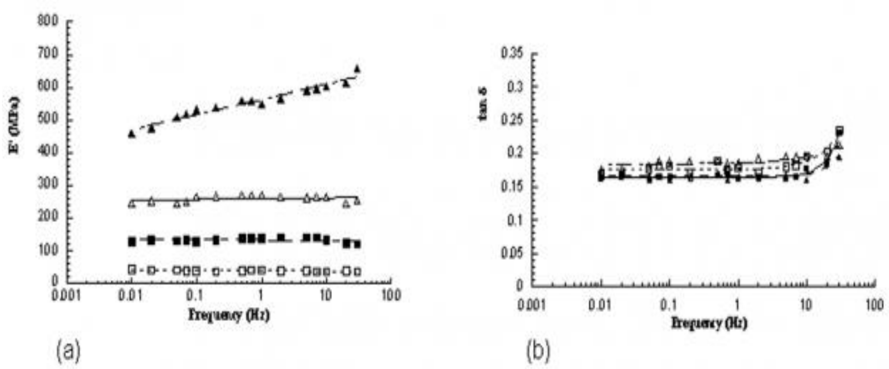

Figure 3: (a) Storage modulus as function of frequency for group A specimens cut along the anterior-posterior direction from intercondilar zone (□) and medial zone (Δ), storage modulus as function of frequency for group B specimens cut along the anterior-posterior direction from intercondilar zone (■) and medial zone (▲); (b) Loss factor modulus as function of frequency for group A specimens cut along the anterior-posterior direction from intercondilar zone (□) and medial zone (Δ), loss factor as function of frequency for group B specimens cut along the anterior-posterior direction from intercondilar zone (■) and medial zone (▲).

Discussion

The components of primary total knee prostheses are designed to cap the cut surfaces of the femur, tibia and patella; in other words, they are "surface replacements" and do not rely on long intramedullary stems for fixation. Hence, joint loads are transferred directly from the cancellous bone of the distal femur to the proximal tibia as it occurs in normal knee. The most common mechanical cause of long-term failure is the loss of component fixation to bone. More commonly, loosening occurs in the tibial component and can result from failure of the trabecular bone. Several studies have measured the migration of proximal tibial component in total knee arthroplasty[29].

This migration, which has been thought to predict the incidence of aseptic loosening, may be a mechanical process rather than a biological one, and, in particular, the mechanism of migration could be due to a fatigue failure of the supporting cancellous bone[30]. This fatigue failure would occur at stress levels above one third of the ultimate compressive strength[14].

Previous studies assumed high ultimate compressive strengths for tibial cancellous bone[31], but for osteoarthritic and rheumatoid cancellous bone, the ultimate compressive strength of resected tibial surface was found to be lower.

Consequently, the mechanical behaviour of tibial spongy bone is crucial for long-term stability of the restored joint. Bone cement can improve the properties of this tissue creating a stronger structure[32], and this is especially important in the case of osteoarthritic, osteoporotic or rheumatoid joints.

Spongy bone highlights a viscoelastic behaviour although it has been usually modelled as a linear elastic material[33]. Time-dependent properties of tibial trabecular bone have been mainly measured through compression tests by varying the strain rate[34,35].

Because spongy bone may be considered as a two phase porous structure, it is well documented that the flow of bone marrow influences its mechanical properties, and the strain rate becomes the dominant factor controlling its behaviour.

In vivo, spongy bone is confined by cortical bone and marrow acts as an incompressible fluid which redistributes the stress to the trabecular network.

In vitro tests are generally performed in unconfined conditions and thus marrow can flow through the pores exiting outside the specimen[34].

Dynamic-mechanical analysis is a technique frequently used in materials science to evaluate the viscoelastic properties of materials. In a earlier study viscoelastic properties of spongy bone have already been measured by nondestructive dynamic-mechanical tests in compression, focusing the attention on the end boundary conditions and specimen geometry[36]. However, in the present study dynamic-mechanical tests using a three-point bending configuration have been considered to assess the viscoelastic

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behaviour of spongy bone and bone-cement system.

It is well known that a three-point bending test is different from a compressive test. The latter gives the possibility to test the specimen along the axis of trabeculae since the stress is uniformly distributed in the orthogonal resistant sections with respect to the applied load, while in the bending the stress varies from negative values (compression) to positive values (tension), reaching maximum values of tension and compression in the external regions of the specimens.

In addition, the proposed configuration (Figure 1d) for testing bone samples has allowed to increase twice the sensitivity of the set-up if compared to a traditional three-point bending test configuration. Moreover, mechanical preconditioning by a stress relaxation[18] or creep is not required. This study has evidenced the time-dependent behaviour of the natural structure and of the bone-cement system through a dynamic three-point bending test.

Figures 2a and 3a clearly show the dependence of the storage moduli on the anatomic site. Accordingly, higher values are measured in the central zone of the medial aspect. This is consistent with previous studies suggesting the highest density of spongy bone in the tibial plateau located in the medial and lateral regions underneath the medial and lateral femoral condyles, respectively[37].

The dependence of the storage modulus on the frequency can be also detected in figure 2a. The slight increase of the storage modulus can be probably due to the marrow and water flow during the loading process. Conversely, for group B specimens a significant increase of the storage modulus is observed as the frequency increases. Such difference can be related to the frequency-dependent behaviour of bone cement[18].

By comparing the storage moduli for bone specimens with those infiltrated with cement, the latter have shown E' values greater than the respective bone specimens without cement. This result may be ascribed to the fact that in bending the stress reaches maximum values in the outer regions of the specimen mainly contributing to the stiffness, and in the case of bone-cement samples these layers are directly infiltrated by the cement.

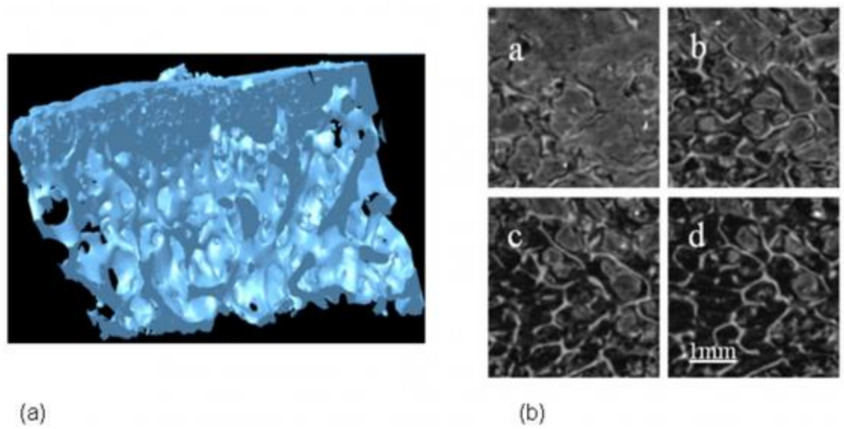

Figure 4: Figure 4. Results obtained from µCT analysis: (a) 3D reconstruction of bone-cement system; (b) images of several bone-cement interfaces at increasing depth.

Since frequency affects the viscous character of materials, frequency influences tan δ, which slightly increases with frequency.

Values of tan δ suggest that at body temperature the bone-cement system shows an ability to dissipate energy that results similar to the natural structure.

PMMA-based cements fill the free space between the prosthesis and bone allowing the stress transfer between the synthetic device and the hosting tissue. The bond between cement and spongy bone is mainly micro-mechanical, therefore, the porosity of bone and the infiltration of the cement into the spongiosa are important prerequisites for the endurance of the synthetic restoration. Furthermore, mCT imaging plays an important role to detect the bone architecture and the state of the bone-cement interface.

The gap between the two materials could be partially due to the cement shrinkage during the polymerisation process. Despite this lack of interface an improvement of mechanical properties of bone has been observed through dynamic-mechanical measurements (Figure 3a).

Anyway, it is worth noting that even though many progresses have been made in cementing techniques and bone cement formulations, nowadays most of research has also been focused on the design of advanced polymer-based scaffolds for bone tissue engineering[38,39,40,41,42,43,44,45,46,47].

Conclusion

Dynamic-mechanical analysis plays an important role to quantify the damping, which is the ability of the natural structure to dissipate mechanical energy. Accordingly, the dynamic-mechanical measurements have shown that the viscoelastic properties of spongy bone vary with direction and region. Moreover, dynamic-mechanical analysis and mCT imaging are a powerful tool to achieve a greater knowledge of the structure-mechanical property-function relationships and, therefore, to improve bone cement injection into the trabecular network.

Our results confirm that bone cement infiltration allows to create a stronger structure enhancing the mechanical performance of spongy bone.

However, the present study has also evidenced the possibility of mapping the viscoelastic properties of the natural structure; thus, the future would be drawn toward this direction in order to design a prosthetic implant, which emulates the biomechanical behaviour of the natural tissues, or a suitable cement, which improves the mechanical properties of spongy bone, preserving the surrounding hosting tissues.

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References
1. Font-Rodriguez DE, Scuderi GR, Insall, JN. Survivorship of cemented total knee arthroplasty. Clinical Orthopaedics and Related Research. 1997; 345: 79-86.
2. Winter DA. Energy generation and absorption at the ankle and knee during fast, natural and slow cadences. Clinical Orthopaedics and Related Research.1983; 175: 147-154.
3. Colley J, Cameron HU, Freeman MAR, Swanson SAV. Loosening of the femoral component in surface replacement of the knee. Archives of Orthopaedic and Trauma Surgery. 1978; 92: 31-34.
4. Yamashita J, Furman BR, Rawls R, Wang X, Agrawal C. The use of dynamic mechanical analysis to asses the viscoelastic properties of human cortical bone. Journal of Biomedical Materials Research (Part B: Applied Biomaterials). 2001; 58: 47-53.
5. Burstein AH, Zika JM, Heiple KG, Klein L. Contribution of collagen and mineral to the elastic-plastic properties of bone. Journal of Bone and Joint Surgery. 1975: 57: 956-961.
6. Privalov PL, Tiktopulo EI. Thermal conformational transformation of tropocollagen. I Calorimetric study. Biopolymers. 1970; 9: 127-139.
7. Finch A, Gardner PJ, Ledward DA, Menashi S. The thermal denaturation of collagen fibers swollen in aqueous solutions of urea, hexamethylenetetramine, p-benzoquinone and tetra-alkylammonium salts. Biochimica et Biophysica Acta (BBA-Protein Structure). 1974; 365: 400-404.
8. Rochdi A, Foucat L, Renou JP. Effect of thermal denaturation on water-collagen interactions; NMR relaxation and differential scanning calorimetry analysis. Biopolymers. 1999; 50: 690-696.
9. Posner AS, Beebe RA. The surface chemistry of bone mineral and related calcium phosphates. Seminars in Arthritis and Rheumatism. 1975; 4: 267-291.
10. Currey JD. Anelasticity in bone and echinoderm skeletons. The Journal of Experimental Biology. 1965; 43: 279-292.
11. Bowman SM, Gibson LJ, Hayes WC, McMahon TA. Results from demineralized bone creep tests suggest that collagen is responsible for the creep behaviour of bone. Journal of Biomechanical Engineering. 1999; 121: 253-258.
12. Cortet B, Marchandise X. Bone microarchitecture and mechanical resistance. Joint Bone Spine. 2001; 68: 297-305.
13. Huiskes R, Weinans H, Grootenboer HJ, Dalstra M, Fudala B, Slooff TJ. Adaptive bone-remodeling theory applied to prosthetic–design analysis. Journal of Biomechanics. 1987; 20: 1135-1150.
14. Hvid I. Trabecular bone strength at the knee. Clinical Orthopaedics and Related Research. 1988; 227: 210-221.
15. Ranawat CS, Flynn WF, Deshmukh RG. Impact of modern technique on long-term results of total condylar knee arthroplasty. Clinical Orthopaedics and Related Research. 1994; 309: 131-135.
16. Ritter MA, Herbst SA, Keating EM, Faris PM, Meding JB. Long term survival analysis of a posterior cruciate retaining total condylar total knee arthroplasty. Clinical Orthopaedics and Related Research. 1994; 309: 136-145.
17. Kuhn KD. In: Bone Cements. Spinger-Verlag, Heidelberg 2000.
18. De Santis R, Mollica F, Ronca D, Ambrosio L, Nicolais L. Dynamic mechanical behaviour of PMMA based bone cements in wet environment. Journal of Materials Science Materials in Medicine. 2003; 14: 583-594.
19. Rho JY. An ultrasonic method for measuring the elastic properties of human tibial cortical and cancellous bone. Ultrasonics. 1996; 34: 777-783.
20. Besdo D, Behrens B, Besdo S, Bouguecha A. Zu problemen bei messungen effektiver schubmoduln von spongiosa-strukturen mit ultraschall-methoden. Biomedizinische Technik. 2004; 2: 848-849.
21. Ebenstein DM, Pruitt LA. Nanoindentation of Biological Materials. Nano Today. 2006; 1: 27-33.
22. Lundkvist A, Lilleodden E, Siekhaus W, Kinney J, Pruitt L, Balooch M. Viscoelastic properties of healthy human artery measured in saline solution by AFM-based indentation technique. In Thin Films: Stresses and Mechanical Properties VI (Mater. Res. Soc. Proc. 436) Gerberich WW, Gao H, Sundgren JE, Baker SP (eds.). Mater. Res. Soc. Boston, MA, USA. 1997, 353-358.
23. Balooch M, Wu-Magidi IC, Lundkvist AS, Balazs A, Marshall SJ, Marshall GW, Seikhaus WJ, Kinney JH. Viscolelastic Properties of Demineralized Human Dentin in Water with Atomic Force Microscopy (AFM)-Based Indentation. Journal of Biomedical Materials Research. 1998; 40: 539-544.
24. Kinney JH, Marshall SJ., Marshall GW. The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature. Critical Reviews in Oral Biology & Medicine. 2003; 14: 13-29.
25. Haque F. Application of Nanoindentation Development of Biomedical to Materials. Surface Engineering. 2003; 19: 255-268.
26. Bembey AK, Oyen ML, Bushby AJ, Boyde A. Viscoelastic properties of bone as a function of hydration state determined by nanoindentation. Philosophical Magazine. 2006; 86: 5691–5703.
27. Yuan Y, Verma R. Measuring microelastic properties of stratum corneum. Colloids and Surfaces B: Biointerfaces. 2006; 48: 6–12.
28. Balooch G, Marshall GW, Marshal SJ, Warre OL, Asif SA, Balooch M. Evaluation of a new modulus mapping technique to investigate microstructural features of human teeth. Journal of Biomechanics. 2004; 37: 1223-1232.
29. Albrektsson BEJ, Ryd L, Carlsson LV, Freeman MAR, Herberts P, Regner L, Selvik G. The effect of a stem on the tibial component of knee arthroplasty: a roetgen stereophotogrammetric study of uncemented tibial component in the

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Freeman-Samuelson knee arthroplasty. Journal of Bone and Joint Surgery. 1990; 72B: 252-258.
30. Taylor M, Tanner KE. Fatigue failure of cancellous bone: a possible cause of implant migration and loosening. Journal of Bone and Joint Surgery. 1997; 79B: 181-182.
31. Cheal EJ, Hayes WC, Lee CH, Snyder BD, Miller J. Stress analysis of a condylar tibial component: influence of metaphyseal shell properties and cement injection depth. Journal of Orthopaedic Research. 1985; 3: 424-434.
32. Windsor RE, Scuderi GR, Moran M, Insall JN. Mechanism of failure of the femoral and tibial components in total knee arthroplasty. Clinical Orthopaedics and Related Research. 1989; 248: 15-23.
33. Deligianni DD, Maris A, Missirlis YF. Stress relaxation behaviour of trabecular bone specimens. Journal of Biomechanics. 1994; 27: 1469-1476.
34. Carter DR, Hayes WC. The compressive behavior of bone as a two-phase porous structure. Journal of Bone and Joint Surgery. 1977; 59-A: 954-962.
35. Linde F, Norgaard P, Hvid I, Odgaard A, Soballe K. Mechanical Properties of trabecular bone. Dependency on strain rate. Journal of Biomechanics. 1991; 24: 803-809.
36. Dong XN, Yeni YN, Les CM, Fyhrie DP. Effects of end boundary conditions and specimen geometry on the viscoelastic properties of cancellous bone measured by dynamic mechanical analysis. Journal of Biomedical Materials Research. 2004; 68A: 573-583.
37. Cameron HU, Hunter GA. Failure in total knee arthroplasty Mechanism, revision and results. Clinical Orthopaedics and Related Research. 1982; 170: 141-146.
38. Gloria A, Russo T, De Santis R, Ambrosio L. 3D fiber deposition technique to make multifunctional and tailor-made scaffolds for tissue engineering applications. Journal of Applied Biomaterials & Biomechanics. 2009; 7: 141-152.
39. Gloria A, De Santis R, Ambrosio L. Polymer-based composite scaffolds for tissue engineering Journal of Applied Biomaterials & Biomechanics. 2010; 8: 57-67.
40. Russo T, Gloria A, D'Antò V, D'Amora U, Ametrano G, Bollino F, De Santis R, Ausanio G, Catauro M, Rengo S, Ambrosio L. Poly(e-caprolactone) reinforced with sol-gel synthesized organic-inorganic hybrid fillers as composite substrates for tissue engineering. Journal of Applied Biomaterials & Biomechanics. 2010; 8: 146-152.
41. De Santis R, Gloria A, Russo T, D'Amora U, D'Antò V, Bollino F, Catauro M, Mollica F, Rengo S, Ambrosio L. Advanced Composites for Hard-Tissue Engineering Based on PCL/Organic–Inorganic Hybrid Fillers: From the Design of 2D Substrates to 3D Rapid Prototyped Scaffolds. Polymer Composites. 2013; 34 (9): 1413-1417.
42. Bartolo P, Domingos M, Gloria A, Ciurana J. BioCell Printing: Integrated automated assembly system for tissue engineering constructs. CIRP Annals - Manufacturing Technology. 2011; 60: 271-274.
43. De Santis R, Gloria A, Russo T, D'Amora U, Zeppetelli S, Dionigi C, Sytcheva A, Herrmannsdörfer T, Dediu V, Ambrosio L. A Basic Approach Toward the Development of Nanocomposite Magnetic Scaffolds for Advanced Bone Tissue Engineering. Journal of Applied Polymer Science. 2011; 122: 3599–3605.
44. Domingos M, Chiellini F, Gloria A, Ambrosio L, Bartolo P, Chiellini E. Effect of process parameters on the morphological and mechanical properties of 3D Bioextruded poly(e-caprolactone) scaffolds. Rapid Prototyping Journal. 2012; 18: 56–67.
45. Patrício T, Domingos M, Gloria A, D'Amora U, Coelho JF, Bártolo PJ. Fabrication and characterisation of PCL and PLA scaffolds for tissue engineering. Rap. Prot. J. 2014; 20 (2): 2014.
46. Gloria A, Russo T, D'Amora U, Zeppetelli S, D'Alessandro T, Sandri M, Bañobre-Lopez M, Piñeiro-Redondo Y, Uhlarz M, Tampieri A, Rivas J, Herrmannsdorfer T, Dediu VA, Ambrosio L, De Santis R. Magnetic poly(ε-caprolactone)/iron-doped hydroxyapatite nanocomposite substrates for advanced bone tissue engineering. Journal of the Royal Society Interface. 2013; 10 (80): 1-11.
47. Russo T, D'Amora U, Gloria A, Tunesi M, Sandri M, Rodilossi S, Albani D, Forloni G, Giordano C, Cigada A, Tampieri A, De Santis R, Ambrosio L. Systematic analysis of injectable materials and 3D rapid prototyped magnetic scaffolds: from CNS applications to soft and hard tissue repair/regeneration. Procedia Engineering. 2013; 59: 233 – 239.

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