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In the trail of a new bio-sensor for measuring strain in bone: Osteoblastic biocompatibility

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ABSTRACT

Fibre Bragg Grating (FBG) is an optical sensor recorded within the core of a standard optical fibre, which responds faithfully to strain and temperature. FBG sensors are a promising alternative to other sensing methodologies to assess bone mechanics *in vivo*. However, response of bone cells/bone tissue to FBGs and its sensing capability in this environment have not been recorded yet. The present study addressed these issues in long-term human osteoblastic cell cultures. Results showed that osteoblastic cells were able to adhere and proliferate over the fibre and, also, the protective polymer coating. RT-PCR analysis showed the expression of Col I, ALP, BMP-2, M-CSF, RANKL and OPG. In addition, cultures presented high ALP activity and the formation of a calcium phosphate mineralized extracellular matrix. Cell behavior over the fibre without and with the coating polymer was similar to that found in cultures grown in standard tissue culture plates (control). In addition to the excellent osteoblastic cytocompatibility, FBGs maintained the physical integrity and functionality, as its sensing capability was not affected through the culture period. Results suggest the possibility of *in vivo* osseointegration of the optical fibre/FBGs anticipating a variety of applications in bone mechanical dynamics.

1. Introduction

Bone tissue is a porous, heterogeneous and anisotropic material that is continually optimized for its load-bearing role by a process of functionally adaptive remodelling by modifying its external geometry and internal structure in accordance to certain mathematical laws. Elucidating the biomechanics of the bone tissue is mandatory to better understand the mechanisms subjacent to normal remodelling and repair processes and also those operating during bone metabolic diseases and injury events. Information regarding the adaptive response of bone to load is necessary to implement prevention programmes of bone loss and to design, evaluate and optimize treatment options of bone repair and regeneration, namely those involving bone engineering strategies.

A variety of methods have been tried to measure bone deformation under load, including strain gauge (Szivek and Gharpuray, 2000), extensometer (Perusek et al., 2001), ultrasound (Vlannderen et al., 2005) and echo tracking (Matsuyama et al., 2006). The strain gauge, a device with electrical resistance varying in proportion to the amount of strain, is the gold standard for measuring bone deformation *in vivo* (Szivek and Gharpuray, 2000; Lanyon et al., 1975). It has been used in a variety of animal studies (De Smet et al., 2008) and, also, few strain measurements in humans have been reported, namely on the tibia during normal and vigorous activity (Burr et al., 1996; Lanyon et al., 1975) and to detect spine fusion (Szivek et al., 2005). However, the strain gauge presents several limitations, hindering its use in the clinical setting to monitor bone deformation during bone metabolic events. It represents a substantial foreign body implant, requires invasive exposure of a site for attachment to the bone surface and only gives information in limited regions of the structure.

To overcome these limitations, other methodologies have been proposed to detect bone deformation under loading with potential *in vivo* application. One of them is based on the optical Fibre Bragg Grating (FBG) technology, as reported by Fresvig et al.

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(2008). They compared strain measurements in an ideal isotropic acrylic material and in a human femur sample to evaluate if bone as a non-homogeneous anisotropic material is suitable for repeated measurements despite the asymmetry, plasticity and non-uniformity of the distribution and transmission of forces, and concluded that FBGs are suitable for dynamic measurements *in vitro*. No significant differences between this methodology and strain gauges were found, suggesting that this technique can be successfully applied in *in vivo* measurements (Fresvig et al., 2008).

Other studies had already accomplished regarding the use of FBGs in bone deformation measurements of orthopaedic and orofacial applications (Carvalho et al., 2002, 2006; Talaia et al., 2007). For instance, Carvalho et al. (2002, 2006) measured strains at a human cadaveric mandible surface caused by static or impact loads on a dental implant and Talaia et al. (2007) assessed bone strains in plated and intact synthetic femurs to analyse the effects of fracture fixation plates. In all of them, comparative loading experiments showed no significant differences between FBGs and strain gauges, in static and dynamic loads (Carvalho et al., 2002, 2006; Talaia et al., 2007). However, FBGs have additional advantages relatively to strain gauges, such as a better dynamic range, have a passive operation and immunity to interference from electrostatic, electromagnetic and radio frequency sources, chemical stability and long-term survivability. In addition, several FBG sensors can be recorded onto a single optical fibre and many fibres can be applied to a structure, providing a mechanism for distributed monitoring (Mohanty et al., 2007). Furthermore, optical fibres in which FGB sensors are recorded are very thin, just 0.125 mm in diameter, meaning that, compared with strain gauges with many soldered seams, would represent a higher comfort for the patient, a smaller risk for infection and the possibility to be used in bone anatomical structures (i.e. curved surfaces) where conventional sensors are not applicable (Fresvig et al., 2008). Hence, it is not unexpected that, in the last years, has been a significant increase on the number of works reporting the application of FBG in biomedicine (Dennison et al., 2008, 2010; Mishra et al., 2010; Ren et al., 2007).

The advantages mentioned above suggest a potential use of FGB sensors as a novel approach to assess bone biomechanics *in vivo*. However, response of bone cells/bone tissue to optical fibre and the sensing capability of the FBGs in contact with bone cells have not been reported yet. In this context, the present study analyses the behaviour of human osteoblastic cells cultured over optical fibre; for this, a preliminary qualitative assay was conducted with MG63 osteoblast-like cells, followed by a detailed evaluation of the proliferation and phenotype behaviour of long-term human bone marrow osteogenic-induced cell cultures. In parallel, the sensing capability of FBG sensors throughout the culture time was assessed.

2. Materials and methods

2.1. Fibre Bragg Grating sensors

An FBG is a periodic modulation of the refractive index of an optical fibre core. If an optical fibre that contains an FBG is illuminated by a broadband light source, only the wavelengths that satisfy the Bragg condition are reflected, being all the others transmitted (Fig. 1A). This condition is given by:

$$\lambda_B = 2n_{eff}\Lambda \quad (1)$$

where λ_B is the reflected Bragg wavelength, n_{eff} the effective refractive index of the fibre core and Λ the periodicity of the refractive index modulation (Othonos and Kalli, 1999).

When the grating is subjected to thermal variations and/or mechanical perturbations the reflected Bragg wavelength changes, according with Eq. (2):

$$\Delta\lambda_B = 2\left(\Lambda\frac{\partial n_{eff}}{\partial\varepsilon} + n_{eff}\frac{\partial\Lambda}{\partial\varepsilon}\right)\Delta\varepsilon_z + 2\left(\Lambda\frac{\partial n_{eff}}{\partial T} + n_{eff}\frac{\partial\Lambda}{\partial T}\right)\Delta T \quad (2)$$

The first term represents the strain dependence on the Bragg wavelength variation and the second term, the thermal effect on the same parameter. Eq. (2) shows that an FBG can be employed as a sensor-element, by monitoring the back reflected light from the Bragg grating, for longitudinal mechanical strain, ε_z, and temperature, T. The sensibility of an FBG to ε_z, is given by:

$$\frac{\Delta\lambda_B}{\lambda_B} = \left\{1 - \frac{n_{eff}^2}{2}\left[p_{12} - \nu(p_{11} + p_{12})\right]\right\}\varepsilon_z \quad (3)$$

where ν is the Poisson's ratio and p_{ij} are the components of the strain-optic tensor. For a typical germanosilicate optical fibre, the expected strain sensitivity around λ_B = 1550 nm is 1.2 pm/$\mu\varepsilon$ (Othonos and Kalli, 1999).

The FBG sensors used in this work were inscribed into a photosensitive single mode optical fibre (FiberCore PS1250/1500) by means of an automated phase-mask interferometer system, using a frequency-doubled argon-ion laser. The Bragg wavelength of the resulting gratings was 1550.00 nm. The optical fibre was constituted by a core with 9.6 μm diameter, made of silica doped with germanium and boron, surrounded by a cladding with 125 μm diameter, made of high purity silica. To provide mechanical resistance, the fibre was provided with a polymer, consisting in a urethane acrylate inner coating followed by an outer epoxy acrylate coating. The overall diameter is 250 μm (Fig. 1B). To inscribe an FBG sensor into the optical fibre, the polymer coating, in the recording region, was previously removed.

The FBGs were submitted to a wet chemical etching in a buffered hydrofluoric acid (HF) solution (40%), during 7.5 min, to create some roughness at the fibre surface, in order to improve cell adhesion. Thereafter, it was determined the sensitivity of the FBGs to strain variations, anchoring a side of the fibre to an unmoved platform and the other side, to a platform mounted on a translation stage with a minimum displacement of 0.01 mm. The translation stage was manually adjusted to induce different strain values ranging from 0 με to 2343.8 με, in 260.4 με steps. These values were calculated taking into account the displacement of the translation stage and the optical fibre length, which was 192.00 mm.

2.2. Osteoblastic cell response to optical fibre

2.2.1. Cell cultures

2.2.1.1. MG63 osteoblast-like cell cultures. MG63 cells were cultured in α-minimal essential medium (α-MEM) containing 10% fetal bovine serum, 50 μg ml^{-1} ascorbic acid, 50 μg ml^{-1} gentamicin and 2.5 μg ml^{-1} fungizone, at 37 °C in a humidified atmosphere of 5% CO_2 in air. For subculture, the cell monolayer was washed twice with phosphate-buffered saline (PBS) and incubated with trypsin–EDTA solution (0.05% trypsin, 0.25% EDTA) for 5 min at 37 °C to detach the cells. Cells were re-suspended in culture medium and were plated (10^4 cell cm^{-2}) over samples of optical fibre without and with polymer coating, which were identified as fibre and polymer, respectively. These samples were previously subjected to an HF treatment, similar to the FBGs, and sterilized by autoclave. Cultures performed in standard tissue culture plates were used as control. Cultures were maintained for 7 days, and characterized by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

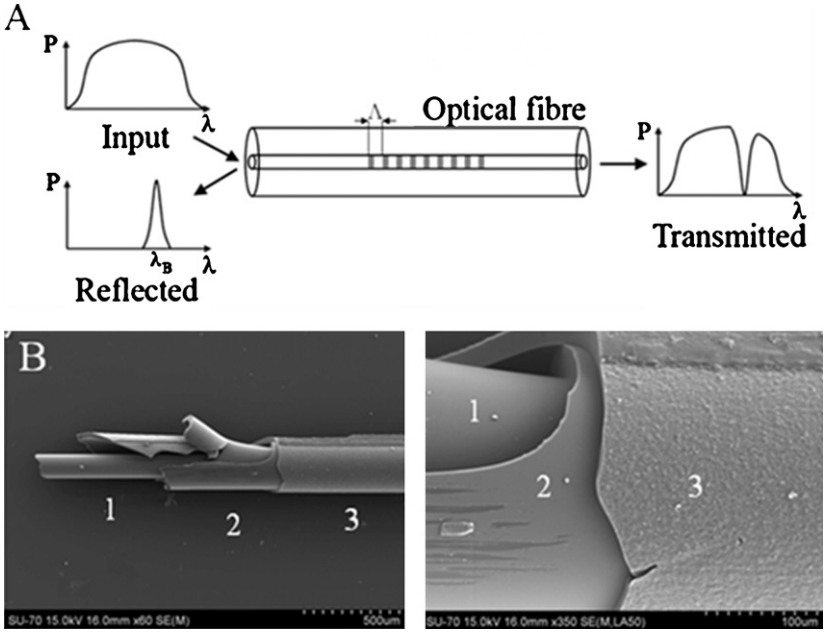

Fig. 1. (A) Schematic representation of an FBG sensor and reflected and transmitted signal; (B) SEM images of the optical fibre tested in the cell culture experiments: (1) Core; (2) Cladding; (3) Polymer.

2.2.1.2. Human bone marrow osteoblastic cell cultures. Human bone marrow, obtained from orthopaedic surgery procedures after a patient (27 years) informed consent, was cultured in the same experimental conditions as those used in the culture of MG63 cells. Cells of the first subculture were plated (10^4 cells cm^{-2}) over samples of fibre and polymer coating, previously subjected to an HF treatment and sterilized by autoclave. After 24 h, the cultures were supplemented with 10 mM β-glycerophosphate and 10 nM dexamethasone, with the culture medium being replaced twice a week. Cultures were maintained for 21 days and assessed at days 7, 14 and 21 for cell viability/proliferation (MTT assay), total protein content, alkaline phosphatase (ALP) activity, ALP and von Kossa staining; also, at day 21, cultures were evaluated for gene expression of osteoblastic proteins and observed by SEM. Cultures performed in standard tissue culture plates were used as control.

2.2.2. Characterization of the cell behavior

2.2.2.1. Cell viability/proliferation. MTT assay (reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrasodium bromide (MTT) to a purple formazan reaction product by living cells) was used to estimate cell viability/proliferation. Cultures were incubated with 0.5 mg ml^{-1} of MTT in the last 4 h of the culture period tested; the medium was then decanted, formazan salts were dissolved with 200 μl of dimethylsulphoxide and the absorbance (*A*) was measured at 600 nm in an ELISA reader.

2.2.2.2. Total protein content and alkaline phosphatase activity. Culture samples were washed twice in PBS, frozen at −20 °C and evaluated at the end of the culture time. The total amount of protein present in the material surface was assayed by the Lowry's method with bovine serum albumin used as a standard. ALP activity was determined in cell-layer lysates (obtained by treatment of the cultures with 0.1% triton in water) and assayed by the hydrolysis of *p*-nitrophenyl phosphate in alkaline buffer solution, pH 10.3, and colorimetric determination of the product (*p*-nitrophenol) at 405 nm. Hydrolysis was carried out for 30 min at 37 °C. Results were expressed as nanomoles of *p*-nitrophenol produced per min per μg of protein (nmol min^{-1} μg $protein^{-1}$).

2.2.2.3. SEM and CLSM observation. For SEM observation, cultures were fixed with 1.5% glutaraldehyde in 0.14 M sodium cacodylate buffer (pH 7.3, 10 min), then dehydrated in graded alcohols, critical-point dried, sputter-coated with gold and analysed in a scanning electron microscope equipped with a X-ray energy dispersive spectroscopy (EDS) microanalysis capability (HR-SEM-SE, Hitachi SU-70; EDS, Bruker QUANTAX 400).

For CLSM, cultures were fixed in 3.7% paraformaldehyde (15 min). Cell cytoskeleton filamentous actin (F-actin) was visualized treating the cells with Alexa Fluor[90] 488 Phalloidin (1:20 dilution in PBS, 1 h). Cultures were counterstained with propidium iodide for cell nuclei labelling. Labelled cultures were mounted in Vectashield® and examined with a Leica SP2 AOBS (Leica Microsystems) microscopy.

2.2.2.4. Gene expression by reverse-transcription polymerase chain reaction (RT-PCR). Cell cultures were assessed at day 21 by RT-PCR for the expression of the housekeeping gene GADPH (glyceraldehyde-3-phosphate dehydrogenase) and the osteoblastic genes Col 1, ALP, BMP-2, OPG, M-CSF and RANKL.

Total RNA was extracted using RNeasy® Mini Kit (QIAGEN) according to manufacturer's instructions. The concentration and purity of total RNA in each sample were determined by UV spectrophotometry at 260 nm and by calculating the $A_{260\,nm}/A_{280\,nm}$ ratio, respectively. RNA, 0.5 μg, was reversely transcribed and amplified (25 cycles) with the Titan One Tube RT-PCR System (Roche), with an annealing temperature of 55 °C. The sequence of the primers used were: GAPDH (5′ primer – CAGGACCAGGTTCACCAACAAGT, 3′ primer – GTGGCAGTGATGGCATGGAC TGT); Col 1 (5′ primer – TCCGGCTCCTGCTCCTCTTA, 3′ primer – ACCAGCAGGACCAGCATCTC); ALP (5′ primer – ACGTGGCTAAGAATGTCA TC, 3′ primer – CTGGTAGGCGATGTCCTTA); BMP-2 (5′ primer – GCAATGGCCT TATCTGTGAC, 3′ primer – GCAATGGCCTTATCTGTGAC); OPG (5′ primer – AAGGAGCTGCAGTACGTCAA, 3′ primer – CTGCTCGAAGGTGAGGTTAG); M-CSF (5′ primer – CCTGCTGTTGTTGGTCTGTC, 3′ primer – GGTACAGGCAGTTG CAATCA); RANKL (5′ primer – GAGCGCAGATGGATCCTAAT, 3′ primer – TCCTCTCCAGACCGTAACTT). After analysis on a 1% (w/v) agarose gel, the RT-PCR products were densitometric analysed with ImageJ

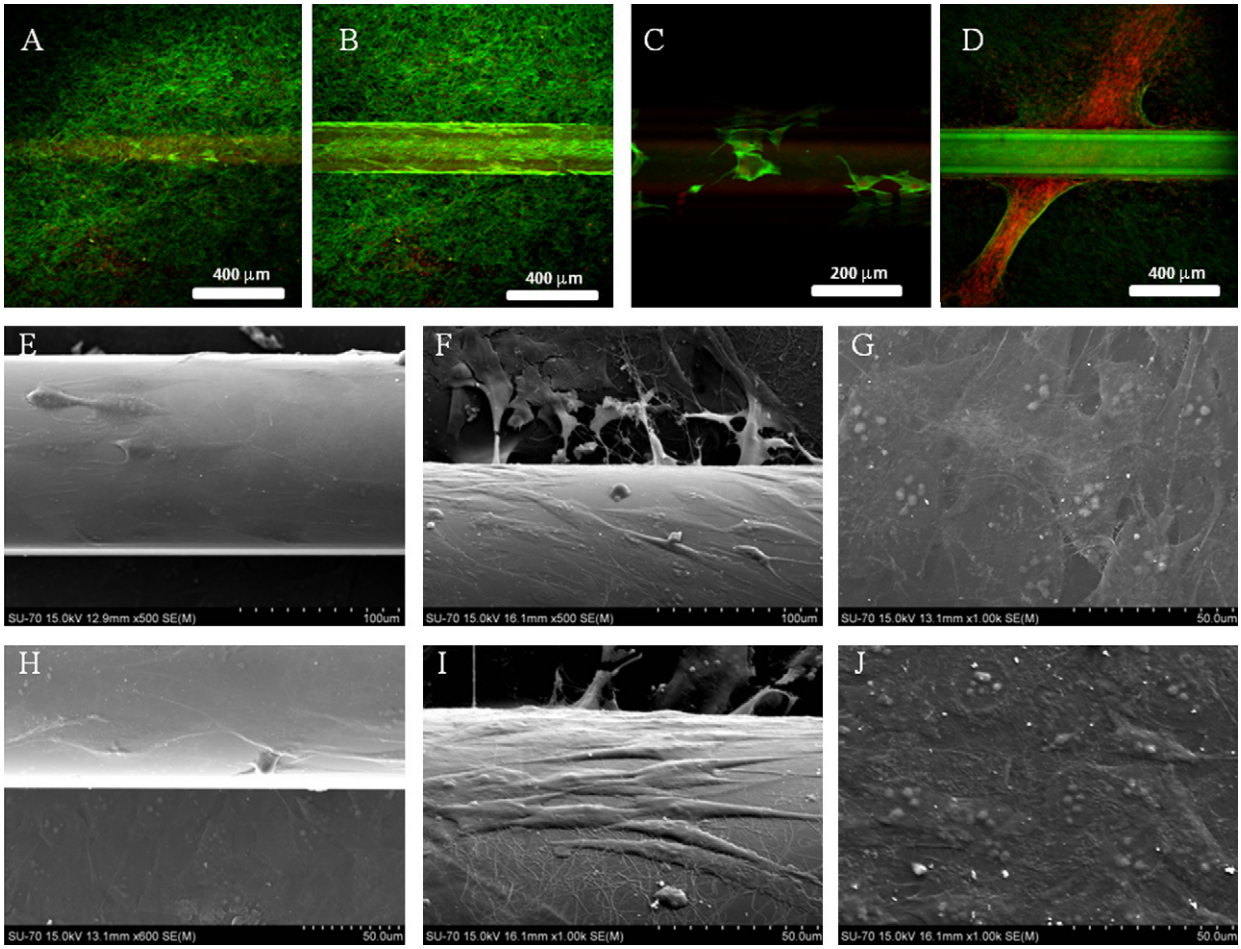

Fig. 2. CLSM observation of MG63 osteoblast-like cells cultured over the fibre and the polymer-coated fibre, at day 7 (A–D). General view of cells cultured over the fibre (A) and polymer-coated fibre (B). Cells growing over the fibre (C) and interface with the culture plate (D). SEM observation of MG63 osteoblast-like cells cultured over the fibre and the polymer-coated fibre, at day 3 (E–J). (E–G): fibre (E, over; F, interface; G, culture plate). (H–J): polymer-coated fibre (H, over; I, interface; J, culture plate).

1.41 software and normalized for the corresponding GAPDH value of each experimental condition.

2.3. Sensing capability of the FBGs during cell culture

During the cell culture, the sensing capability of the FBGs was tested at defined time-intervals. The readings from FBGs immersed in culture medium in the absence of cells served as control. Acquisition data was performed at days 1, 4 and 7 for MG63 cell cultures and at days 1, 6, 13 and 21 for human bone marrow cell cultures. Data acquisition was made at 1 sample/s, during 120 s, using a sm125 interrogation system from Micron Optics, with 10 pm of wavelength resolution. The results presented here are the average value for each 120 s measured.

As the FBGs are also sensitive to thermal variations, the data acquisition was performed in a stabilized temperature (culture conditions, 37 °C, 5% CO_2/air).

The integrity of the sensor was assessed collecting the FBG spectrum before and after the experiments.

2.4. Statistical analysis

Triplicate experiments were performed. The results are shown as the arithmetic mean ± the standard deviation (±SD). Analysis of the results was carried out using the Student's *t*-test, with a significance level of $p < 0.05$.

3. Results

3.1. Osteoblastic cell response to FBGs

3.1.1. MG63 osteoblast-like cells

SEM and CLSM appearance of MG63 osteoblastic-like cells seeded over the fibre without and with polymer coating are shown in Fig. 2. Cell proliferation was observed over the fibre and in its vicinity, with no apparent signs of cytotoxicity. Cells appeared well spread, with an elongated morphology and extensive cell-to-cell contact. After few days, the fibre was completely covered by cell multilayers and, also, exuberant cell growth at the interface with the culture plate was observed. The coating polymer presented a similar behaviour.

3.1.2. Human bone marrow osteoblastic cells

Results regarding the proliferation and function of human bone marrow osteoblastic cells cultured over the fibre and the polymer coating are shown in Figs. 3–5. Cell response was similar in control cultures (grown in standard tissue culture plates) and those performed over the fibre and the polymer coating. Cells proliferated throughout the 21 days incubation period (Fig. 3A), and ALP activity increased significantly during the second week (Fig. 3B). On histochemical assays, cultures showed a typical pattern of cell growth with the presence of cell clusters which stained intensively for ALP (Fig. 3C). The fibre and the polymer became fully incorporated within the cellular organization of the osteoblastic

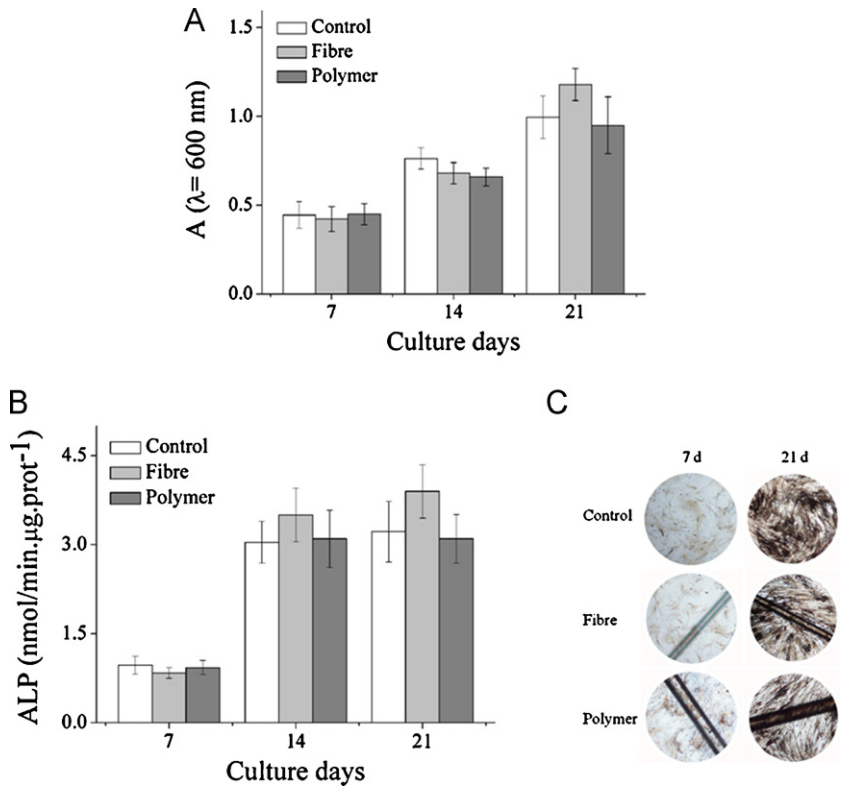

Fig. 3. Viability/proliferation (A), ALP activity (B) and macroscopic view of ALP staining (C) of human bone marrow osteoblastic cells cultured over the fibre and the polymer-coated fibre. Cultures performed in standard tissue culture plates were used as control.

cultures, with evident ALP production and matrix mineralization over the material and at the interface with the culture plate. Calcium phosphate deposits were detected already at day 14 (not shown) and increased with culture time, with 21 day cultures showing strong positive von Kossa reactions (Fig. 4). SEM observation at day 21 presented evident calcium phosphate matrix mineralization over the fibre and polymer and in their vicinity. Fig. 4 shows representative images of control cultures and those performed over the fibre. Mineral deposition was mainly associated with the high density cell clusters, as evident by the mapping of the distribution of the calcium phosphate deposits. Organization of the cell layer and pattern of matrix mineralization over the fibre and at the interface with the culture plate was similar to that of control (Fig. 4). In addition, RT-PCR analysis at day 21, Fig. 5, shows an identical expression profile of COL I, ALP, BMP-2, OPG, M-CSF and RANKL in control cultures and

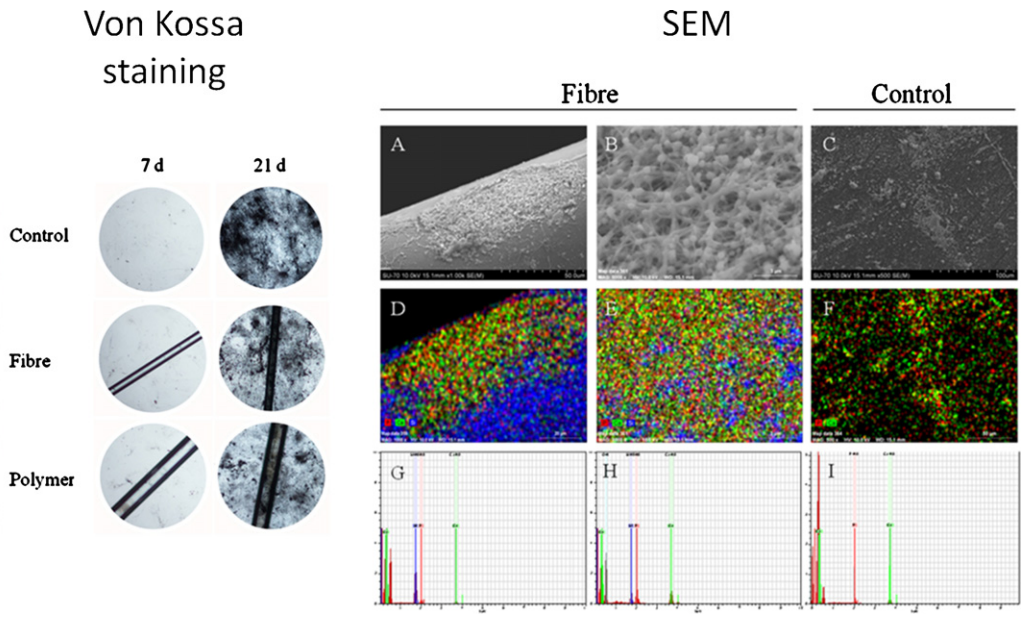

Fig. 4. Macroscopic view of von Kossa staining of human bone marrow osteoblastic cell cultures performed over the fibre and the polymer-coated fibre. Cultures grown in standard tissue culture plates were used as control. SEM appearance of human bone marrow osteoblastic cell cultures performed for 21 days over the fibre. Cultures grown in standard tissue culture plates were used as control. (A–C) SEM images; (D–F) Ca and P distribution; (G–I) X-ray spectra.

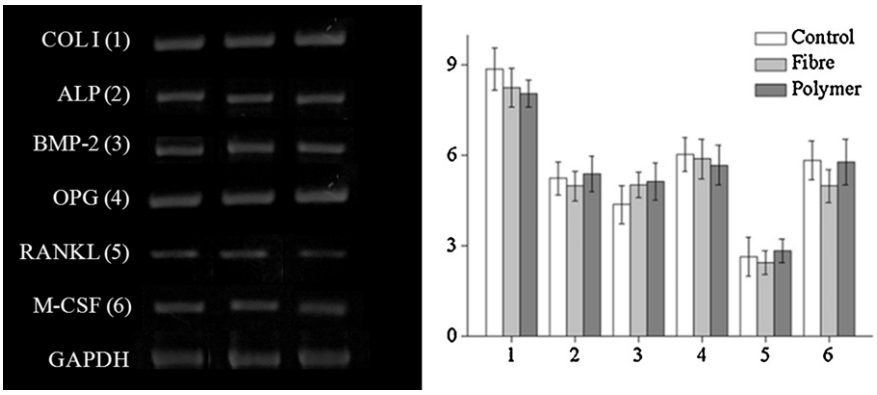

Fig. 5. RT-PCR analysis of human bone marrow osteoblastic cell cultures performed for 21 days over the fibre and the polymer-coated fibre. Cultures grown in standard tissue culture plates were used as control.

those performed in the presence of the fibre and the coating polymer.

3.2. Sensing capability of the FBGs during cell culture

Fig. 6A depicts the reflected Bragg wavelength as function of the strain applied with the translation stage. The sensitivity coefficient of the FBG to deformation was calculated by the slope of the linear fit to the experimental data and the value found was (1.35 ± 0.01) pm/$\mu\varepsilon$. As the interrogation system has a wavelength resolution of 10 pm, it can measure strain variations down to $\pm 7.4\ \mu\varepsilon$.

Fig. 6B shows the strain measurements obtained with the FBGs incorporated in human osteoblastic cell cultures throughout the 21 day incubation time and, also, immersed in culture medium in the same experimental conditions (control). In both situations, strain measurements were under the sensitivity limit of the system, as mentioned above. The spectra of the grating, before and after the tests, are presented in Fig. 6C.

4. Discussion

FBGs are a promising alternative to other sensing methodologies to assess bone mechanics *in vivo*. Studies performed in *in vitro* loading experiments (Carvalho et al., 2002, 2006; Fresvig et al., 2008; Talaia et al., 2007) showed that compared with strain gauges, the output signals recorded with FBGs were similar, but with a significantly higher signal to noise ratio (SNR), being this higher sensitivity a clear advantage when measuring small strains. However, bone compatibility of optical fibre and the sensing capability/integrity of FBGs in contact with bone cells have not been addressed yet.

The present study reports the proliferation and differentiation behaviour of human osteoblastic cells cultured over optical fibre without and with polymer coating. A preliminary experiment showed that MG63 osteoblast-like cells were able to attach, spread and proliferate over the fibre samples and no signs of cell toxicity were noticed over the surface, the interface with the culture plate and surrounding cell culture, as shown by CLSM and SEM observation. In a more detailed experiment, biocompatibility of these samples was addressed with human bone marrow cells cultured in experimental conditions known to favour osteoblastic differentiation, i.e. in the presence of ascorbic acid, β-glycerophosphate and dexamethasone (Coelho and Fernandes, 2000).

Bone marrow osteoblastic cells seeded over the fibre and the protective polymer presented similar behaviour to that of control cultures (performed on standard tissue culture plates). Cultured cells proliferated during the 21 day incubation time and synthesized ALP, with a significant increase during the second week, suggesting an osteoblastic differentiation pathway (Aubin and Triffitt, 2002). In addition, cell layer exhibited a typical pattern of cell growth, with the presence of exuberant cell clusters that stained intensively for ALP and, by day 21, presented evident matrix mineralization, as shown by positive von Kossa staining and SEM observation with cell-associated calcium phosphate deposits. This behaviour is in line with that established for the development of the osteoblast phenotype (Aubin and Triffitt, 2002).

PCR analysis of the cultures performed in the presence of the fibre and the protective polymer showed the expression of the osteoblastic markers COL I, ALP and BMP-2. Collagen type I is the most abundant extracellular bone matrix, being considered an early bone differentiation marker, which has a role in osteoblast differentiation and also controls the nucleation site and growth space of hydroxyapatite (Aubin and Triffitt, 2002). Also, ALP gene expression represents a frequently used early marker for osteogenic differentiation, as ALP has a determinant role in the mineralization of the extracellular collagenous matrix, by providing phosphate ions that, with calcium ions, are used in the formation of cell-mediated

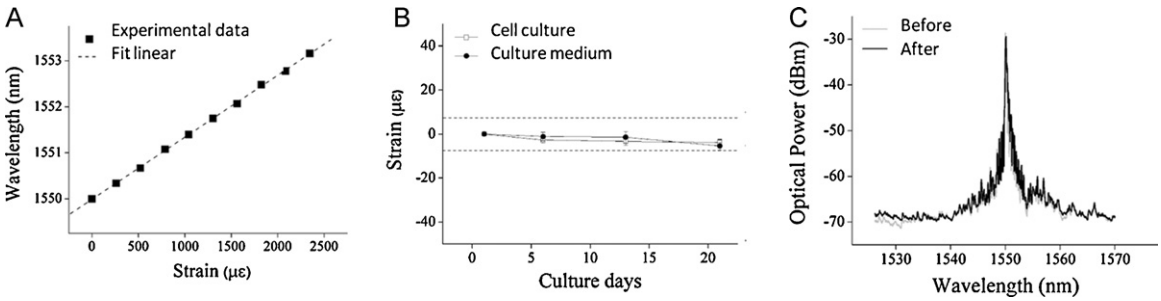

Fig. 6. (A) Strain sensitivity of the HF pre-treated FBG ($R = 0.99982$; $E(y) = 10$ pm); (B) Strain measurements obtained with the FBGs during the culture time; (C) Reflection spectrum of an FBG before and after cell culture testing.

calcium phosphate deposits (Aubin and Triffitt, 2002). BMPs are members of the TGF-beta superfamily of cytokines that affect bone formation by inducing osteoblast differentiation and maintenance of mature osteoblasts (Aubin and Triffitt, 2002; Yamaguchi et al., 2000). Human bone marrow cell cultures growing over FBGs and in its vicinity also expressed M-CSF, RANKL and OPG genes in a way similar to control cultures. This is a relevant issue, as osteoblastic cells, in addition to being responsible for the bone formation events, are essential modulators of the osteoclastogenesis through the production of a variety of molecules (Matsuo and Irie, 2008; Datta et al., 2008). Among them, M-CSF and RANKL, acting on RANK and c-Fms receptors on osteoclast cells, respectively, are necessary for osteoclast survival, differentiation and activation (Boyle et al., 2003). On the other hand, OPG is a decoy receptor that binds to RANKL, blocking RANKL from binding to the RANK receptor on osteoclasts, inhibiting osteoclastogenesis (Boyce and Xing, 2008). Therefore, the present results suggest that osteoblastic cells contacting with optical fibre present a normal behaviour regarding paracrine pathways of osteoblast/osteoclast communication, essential for bone development and remodelling.

Optical fibre is composed by a core made of silica doped with germanium and boron, surrounded by a silica cladding, which explain the observed osteoblastic compatibility. The clinical significance of silica in the bone tissue is well known since the early studies of Carlisle (1970) and the biocompatibility of glasses and glass ceramics bone materials is well documented (Vallet-Regí et al., 2003). In line with this, a previous study reported that an optical fibre made with inert silica glass was shown to be biocompatible following implantation in the paravertebral muscles of New Zealand rabbits (Yang et al., 2003). Regarding the coating polymer, consisting in a urethane acrylate inner coating followed by an outer epoxy acrylate coating, several studies suggest a low cytotoxicity profile of these materials (Quinn et al., 1995; Kanie et al., 2009; Pereira et al., 2010).

In addition to the excellent cytocompatibility, results showed that FBGs maintained the physical integrity and functionality, as its sensing capability was not affected throughout the 21 day cell culture period. In fact, both reflection spectra, before and after the experiments, maintained the same pattern (Fig. 6C). As the culture medium simulates many of the features of the extracellular fluid, this observation suggests the functionality of FBGs in *in vivo* conditions. However, all strain measurements were under the sensitivity limit of the system, suggesting that the cell layer growing over the sensor was not able to stretch it enough, which is not surprising considering the *in vitro* environment with a limited number of cell layers lacking tri-dimensional structure.

5. Conclusion

The optical fibre without and with polymer coating exhibited an excellent osteoblast cytocompatibility, as assessed with human bone marrow cells cultured in osteogenic-induced conditions. Osteoblasts were able to adhere and proliferate over the fibre and the protective polymer coating. Cultures presented a representative proliferation/differentiation behaviour with a typical osteoblast gene expression profile, ALP synthesis and matrix mineralization. In addition, cultures showed a normal expression of M-CSF, RANKL and OPG, factors involved in the osteoblast–osteoclast communication, essential in the bone regeneration and remodelling.

The cytocompatibility of the optical fibre and, consequently, FBGs, suggest the possibility of its osseointegration that, together with the maintenance of the physical and functional integrity of the sensor in culture conditions, anticipates a variety of *in vivo* applications in bone mechanical dynamics.

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