1-1-2016

**Mechanical fatigue performance of PCL-chondroprogenitor constructs after cell culture under bioreactor mechanical stimulus**

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Abstract
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Keywords
cell, fatigue, culture, under, bioreactor, stimulus, performance, pcl, chondroprogenitor, constructs, after, mechanical

Disciplines
Engineering | Science and Technology Studies

Publication Details

Authors
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This journal article is available at Research Online: https://ro.uow.edu.au/eispapers/5250
Mechanical fatigue performance of PCL-chondroprogenitor constructs after cell culture under bioreactor mechanical stimulus

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Abstract

In tissue engineering of cartilage, polymeric scaffolds are implanted in the damaged tissue and subjected to repeated compression loading cycles. The possibility of failure due to mechanical fatigue has not been properly addressed in these scaffolds. Nevertheless, the macroporous scaffold is susceptible to failure after repeated loading-unloading cycles. This is related to inherent discontinuities in the material due to the micropore structure of the macro-pore walls that act as stress concentration points. In this work, chondrogenic precursor cells have been seeded in Poly-ε-caprolactone (PCL) scaffolds with fibrin and some were submitted to free swelling culture and others to cyclic loading in a bioreactor. After cell culture, all the samples were analyzed for fatigue behavior under repeated loading-unloading cycles. Moreover, some components of the extracellular matrix (ECM) were identified. No differences were observed...
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**Keywords:** Poly-ε-caprolactone, fatigue testing, bioreactor, biomaterials, fibrin

**Introduction**

Articular hyaline cartilage is a tissue that provides low friction and load bearing features to the hips and knees. Its mechanical properties are a result of an organized ECM, containing proteins like sulphated glycosaminoglycans, which allow high water uptake \(^1\)\(^-\)\(^3\). Cartilage is an avascular tissue and their single cells, the chondrocytes, show a slow metabolism, as a consequence of limited nutrients and oxygen. Given its inability to self-repair after injury or aging, tissue engineering therapies are a valuable option for the regeneration of cartilage.

These strategies consist on growing cells in 3D native-like environments - e.g. hydrogels or polymeric scaffolds - and providing adequate stimulus, typically through growth factors and other soluble molecules, guiding the process of cell differentiation \(^4\). In the case of chondrogenic differentiation, in vitro cultures are performed for long periods – usually 28 days or more \(^5\)\(^,\)\(^6\). It has been demonstrated that bioreactors that apply dynamic loading can improve differentiation, through control of both the applied mechanical loads and the cell state of differentiation. Moreover, these strategies can also serve as models for understanding the processes involved in cartilage ECM remodeling. This process can be monitored by the analysis of specific ECM markers, and also through the increase of the elastic modulus.

The cyclic mechanical loads applied to the scaffolds leads to material fatigue. Such effect, known to occur in biomaterials \(^7\), has been rarely assessed for the constructs combining the
scaffold and the ECM produced by the cells inside the pores. This can actually be quite relevant since the development of ECM can modify the fatigue resistance of the scaffold, being more representative of the mechanical scaffold performance in vivo. Indeed, any factor limiting water permeation through the material will contribute to apparent elastic modulus and fatigue behavior. Thus, the ECM can play a role in fatigue resistance, due to its ability to retain water. Fatigue analysis can thus provide better insight and new information not only on the mechanical performance of the constructs but also on the ECM integration with the scaffold surfaces.

Fatigue behavior of materials can be influenced by several factors such as thermal and mechanical loading history, environmental conditions, polymer composition and other aspects of stress-strain constitutive behavior. Several mathematical models were developed to predict fatigue behavior, mostly in metallic materials and composites, during load-recovery cycles such as Coffin–Manson, Smith-Watson-Topper (SWT) or Morrow models. The later one is based on the evolution of the plastic strain energy density that can be physically interpreted as the distortion energy associated to the change in shape of a volume element and can be related to failure, in particular under conditions of ductile behavior. This model has been successfully applied to describe the poly-ε-caprolactone (PCL) fatigue behavior under cyclic mechanical loading and to assess the influence of water and poly(vinyl alcohol) (PVA) to the PCL macroporous structure.

Fibrin is a hydrogel that not only can provide mechanical resistance by water retention but it is also adhesive to cells, resembling some aspects of a pericellular matrix. It has been found that in the presence of cyclic mechanical loading, fibrin hydrogels induce higher expression of chondrogenic markers than non-adhesive hydrogels, in mesenchymal stem cell culture. Further, fibrin within PCL scaffolds has a minor impact on fatigue behavior as compared with the effect of the water. Therefore fibrin hydrogels can be of interest for cartilage tissue engineering.

In the present work, chondrogenic precursor cells were seeded in fibrin hydrogels formed inside PCL macroporous scaffolds and cultivated in chondrogenic medium. To simulate the physiological mechanical environment of the upper zones of cartilage, the culture was performed in a bioreactor able to produce cyclic compression by displacement control. The fatigue behavior of the constructs was analyzed overtime, and the contribution of the ECM produced during cell cultivation on the properties observed is discussed.
Materials and methods

**Materials:** Poly-ε-caprolactone (PCL, 43-50 kDa) and 1,4-dioxan were purchased from Sigma-Aldrich. Poly(ethyl methacrylate) (PEMA - Elvacite 2043) spheres (mean diameter of 200 μm) were purchased from Lucite. Fibrinogen from human plasma 50-70% protein (≥80% of protein is clottable) and thrombin from human lyophilized plasma powder, ≥2,000 NIH units/mg protein (E1%/280, 18.3) were purchased from Sigma-Aldrich, as well as L-Proline, TGF-β1, ascorbic acid, dexamethasone, TriReagent and isopropanol. Coagulation factor XIII was purchased from Merck. ITS, DNAse I and PicoGreen DNA quantification kit were purchased from Invitrogen. iScript kit for reverse transcription was purchased from Biorad, Power SYBR™ Green PCR Master Mix for real-time PCR was purchased from Applied Biosystems.

**Sample preparation:** PCL (Sigma-Aldrich) scaffolds were prepared as previously described. Briefly, PCL was dissolved in dioxane (25% w/v) and this solution was mixed with PEMA (Lucite) microspheres (1:1 w/w). Then, the mixture was placed in Teflon Petri dishes and submerged in liquid nitrogen for a minute. Dioxane (Sigma-Aldrich) was extracted from the frozen plates with ethanol at -20 °C for three days, changing ethanol every day. Porogen leaching was performed in ethanol at 40 °C for one day. The porous samples were cut into cylinders with 5 mm diameter and a thickness of approximately of 2 mm. Further leaching for each cylinder was performed in ethanol at 40 °C for nine days, changing ethanol daily, in order to assure complete removal of porogen.

**Sample Characterization:** Sample morphology and gel structure was assessed by scanning electron microscopy using a JEOL JSM-5410 apparatus equipped with a cryogenic device. Images were taken at an accelerating voltage of 10 kV. Samples were previously immersed in water during 24 h and then frozen at -80 °C. Then, the samples were cryo-fractured and water was sublimated during 40 min before coating with a gold thin layer.
Cell culture in expansion medium: PCL scaffolds were sterilized by gamma radiation at 25 kGy. An immortalized cell line with chondrogenic potential from murine bone marrow was used - KUM5 (Riken Cell Bank)\textsuperscript{15}. Cells were expanded in DMEM 4.5 g/L glucose (Gibco) with 10% FBS and 1% penicillin/streptomycin. After passage 25, 2·10\textsuperscript{5} cells were trypsinized, resuspended in 50 µl of medium and seeded in the PCL scaffolds by injecting with a chromatography syringe.

Another group of PCL scaffolds was seeded with cells resuspended in 25 µl of 5 U/ml thrombin solution with 20 mM CaCl\textsubscript{2} (supplemented with coagulation Factor XIII, final concentration 70 µM). The cells were directly injected in the scaffold with a bowel chromatography syringe at a density of 10\textsuperscript{6} cells/scaffold. Simultaneously, 25 µl of filter-sterile fibrinogen were injected using another syringe. Scaffolds were held for 1 h to allow coagulation of fibrin and then submerged in the same culture medium used for expansion, in standard culture plates (48 wells). Cell culture was performed for 21 days at 37 °C and 5% CO\textsubscript{2}.

The samples were observed in cryoSEM to analyse the fibrin effects on cell adhesion. After 1 day of cultivation, cells and scaffolds were fixed in glutaraldehyde 2.5 % for 1 h at 4 °C. CryoSEM was performed in a JEOL JSM-5410 equipment as previously indicated.

To estimate cell proliferation, three replicas were taken at days 0, 3, 6, 14 and 21, digested with proteinase K and the DNA content was quantified with a Picogreen kit, following manufacturer instructions, using a standard curve obtained using the lambda DNA provided in the kit.

Cell culture in bioreactor: PCL scaffolds were sterilized as described previously, and cells were expanded and seeded with fibrin in the scaffolds as described above, with exception of the cell number, which in this case was 10\textsuperscript{6} per scaffold. For bioreactor culture, the inoculated constructs with the fibrin clot were submerged in chondrogenic medium: DMEM 4.5 g/l glucose containing L-proline 50 µg/ml, ascorbic acid 50 µg/ml, dexamethasone 10\textsuperscript{-7} M, ITS+Premix 1%, penicillin/streptomycin 1% and TGF-β1 10 ng/ml. In these experiments, only the PCL/fibrin scaffolds were employed.
Constructs with cells were kept until day 14th in free-swelling conditions\textsuperscript{16,17}. Then, half of the samples were submitted to cyclic compression for 28 days in the bioreactor and the remaining samples were kept in free-swelling conditions. The home-made bioreactor can hold multiple samples under loading by Teflon cylinders, in a 48 well plate. Its configuration allows taking the plate under laminar hood for sample acquisition, changing the medium manually, and placing it in standard cell incubator. The loading profile was: 30 minutes with onset strain of 15\% at a frequency of 1 Hz, and 90 minutes of stillness. The medium was changed every 3 days during stillness periods.

\textit{Fatigue trials:} Following cell culture, mechanical experiments were performed on both the bioreactor–loaded and free-swelling samples, using a Shimadzu AG-IS universal testing machine, in compression mode, at a test velocity of 1 mm-min\textsuperscript{1} and room temperature. In fatigue experiment, samples were submitted to a compressive-strain cycle load up to 1000 cycles to a maximum strain of 15\% per cycle, which is typically the strain range of interest as it is considered to be the maximum magnitude of physiological deformation suffered by articular cartilage \textsuperscript{18,19}. Strain deformation was measured by machine cross-head displacement and mechanical stress and strain parameters were obtained as an average of five samples (n=5). All mechanical experiments were performed with the sample immersed in deionized water. Elastic moduli were calculated from data of each first cycle. These values and the fitting parameters of the Morrow model (described below) are presented as the mean ± standard deviation and were analyzed using a one factor ANOVA statistical study. Differences are considered significant for p<0.05.

\textit{Real-time PCR:} In order to identify the cell expression of characteristic markers of several ECM components, quantitative real-time PCR was performed. PCL samples were cultured with the same medium and cell seeding conditions - including fibrin encapsulation – as those for mechanical analysis. Samples were collected after 1 and 14 days under free-swelling conditions. After 14 days, half of the samples were submitted to cyclic compression for 28 days in the bioreactor and the other half were kept in free-swelling conditions. Samples from both groups were taken (N=3). The total RNA was isolated using 1ml TriReagent\textsuperscript{20}, as follows: 0.1ml 1-bromo-3-chloropropane was added and the aqueous phase containing the RNA was taken by
centrifugation at 12000 g at 4 °C for 15 min and mixed with 500ml isopropanol. After an incubation period of 10 min at room temperature and centrifugation for 10 min at 12000 g, the pellets were washed twice with 1 ml ethanol 75% and dried in a fume hood for 10 min at room temperature. The pellets were dissolved in 30 µl of RNAse-free water and treated with DNAse I to eliminate genomic DNA for 30 min. The amount and purity of total RNA was determined in a Nanodrop 1000 spectrophotometer (Thermo-Fisher). cDNA was synthesized with the iScript reverse transcription kit, following the manufacturer protocol.

For real-time PCR, the primers were purchased from Stabvida and their sequences are provided in Table 1. The expression of collagen type I and collagen type II was quantified. The amplification was carried in a CFX96 Real-time system, C1000 thermal cycler (Biorad), with the following protocol for all genes: amplification was performed for 40 cycles, each one consisting in denaturing at 95 0C for 5 s and annealing /extending at 60 0C for 40 s. The Ct values were obtained with Biorad CFX Manager software and used for expression analysis of target genes using the \(2^{-\Delta\Delta Ct}\) calculation method \(^{21}\), with β-actin as reference gene. Results are presented as the mean ± standard deviation and were analyzed using a one way ANOVA statistical study. Differences are considered significant for p<0.05.

### Table 1

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### Results and Discussion

Poly-ε-caprolactone (PCL) exhibits a porous architecture with macropores ranging from 120 up to 200 µm obtained from the leaching of porogen spheres. SEM pictures show that the macropores are well interconnected with large pore throats and in addition the pore walls are microporous with small pores that result from dioxane crystals formed during the freeze extraction process (figure 1 a). A more detailed analysis of the pore dimensions and the effect of processing on macro- and micro-pore structure can be found in reference \(^{22}\). This double porosity of the PCL samples favors scaffold permeability to nutrients and cell metabolic waste products and can be used to retain different active components \(^{23-25}\). However, apparent scaffold stiffness
becomes smaller than in similar sponges lacking microporosity \textsuperscript{26,27}. Further, when fibrin is added to PCL scaffolds, the fibers become attached to the porous matrix walls (figure 1 b). When cells are seeded into these scaffolds (with and without fibrin) for 24h, they interact and integrate well with the polymer matrix, adhering to the polymer scaffold surface and displaying a flattened morphology with conical protrusions (figure 1 c and d).

\textbf{Figure 1}

Cell attachment is different in scaffolds with and without fibrin. While cells seeded in the scaffold without fibrin attach to pore walls and spread resembling the culture in 2D monolayer \textsuperscript{28}, cells in fibrin matrix take a round shape with adhesion points in 3D.

\textit{Cell culture on PCL scaffolds in non-differentiation medium}

Initially, the number of cells is similar for both conditions (Fig. 2), but for subsequent days, fibrin containing scaffolds present fewer cells. However, while the cell density in scaffolds without fibrin seems reach a plateau towards the end of the cultivation period, cell proliferation in the presence of fibrin accelerates at a later stage such that the number of cells in both conditions eventually tends to match. According to statistical analysis, the difference is not substantial between the two groups at de 21\textsuperscript{st} day, being pronounced through time for each group and between them in the rest of the time. Considering that the fibrin gel is mostly degraded by day 21 in the presence of fibrinolytic cell activity\textsuperscript{29}, it can be speculated that fibrin limits cell growing until it is completely degraded. The effect of fibrin clot in our “in vitro” experiments must be analyzed since it mimics the physiological conditions observed when a scaffold is implanted in a cartilage defect combined with microfracture of subchondral bone. Bleeding in the zone of the implant originates a clot which fills the scaffold pores, acting as migration path for mesenchymal stem cells coming from subchondral bone. \textsuperscript{26,30,31} On the other hand it has been demonstrated that fibrin encapsulation play a positive role in mechanotransduction \textsuperscript{13,14}, possibly simulating the necessary pericellular environment for mechanosensitive cells \textsuperscript{16} Therefore, all the assays for differentiation in this work were performed in the presence of fibrin.
Mechanical behavior

The differential 3D structure affects cell growing rate over time, and can affect the way mechanical loads are sensed by the cells as well, through different cytoskeleton configurations. The fatigue behavior of PCL with fibrin constructs was analyzed after cell culture, both under free-swelling and loading conditions. The representative mechanical hysteresis loops obtained are presented in figure 3. Lower hysteresis effects as well as lower maximum stress are noticed in each cycle for the samples under static and dynamic cell culture (figure 3 a and b). The polymer scaffolds elastic modulus \( (E) \) was determined for the first cycle at \( \varepsilon = 5\% \) and it was observed that it increases with the incorporation of fibrin. The elastic moduli of the PCL+Fibrin constructs before and after cell culture were compared. Both PCL+Fibrin groups show a slight increase of the elastic modulus after cell cultivation. The elastic moduli after cell culture were also compared with data reported for PCL filled with a PVA gel, that hardens by freeze/thawing cycles and serves as mechanical model of growing tissue. The elastic modulus after cell culture is similar to the highest values obtained using PCL+PVA with 6 cycles of freeze-thawing. No statistical difference in elastic modulus is observed between cell culture samples under free-swelling and cyclic loading conditions. Nevertheless, it was observed a decrease of the maximum stress after each cycle, which suggests that the material undergoes permanent deformation during the first ~50 loading cycles, then reaching a stable plateau (figure 3d).
Morrow energy model: plastic strain energy density-life model

Under cyclic loading, the plastic strain energy per cycle is considered a measure of the amount of fatigue damage per cycle. The amount of plastic strain and the energy absorbed during cyclic loading by the material has been postulated as a basis for failure analysis. The relation between plastic strain energy density and the fatigue life can be expressed as

\[ N_f^m W_p = C \]  

(1)

where \( W_p \) is the overall equivalent behavior similar to plastic strain energy density; \( N_f \) is the fatigue life and \( m \) and \( C \) are the fatigue exponent and coefficient, respectively.

Fatigue life for PCL scaffolds immersed in water and after cell culture (free-swelling and in bioreactor) submitted to cyclic compressive loading is presented in figure 4. Experimental data were fitted according to equation 1 to evaluate material response to cyclic mechanical loading before ample collapse with \( R > 0.98 \). Fitting results are presented in figure 4 as solid lines and the fitting parameters are represented in table 2. A decrease of fatigue exponent (slope of figure 4a) and coefficient (y-intercept) was observed for the samples submitted to cell culture (free-swelling and in bioreactor) and posterior mechanical compressive cyclic experiments (table 2), which indicate the decrease in sample mechanical hysteresis and consequently lower energy loss observed in figure 3 (a and b). According to statistical analysis, the slopes are significantly different between the PCL samples (with and without fibrin), and the samples obtained after cell culture under free-swelling and load bearing conditions, meaning that a larger number of cycles are needed after cell culture to dissipate the same energy. The constructs obtained after cell culture thus have an increased resistance to fatigue. However, the slope is higher in samples obtained in this work after cell culture than those reported for PCL+PVA.

Figure 4

Table 2
PCL constructs mechanical life cycle performance was calculated according to the fitting parameters obtained by Morrow’s model (table 2) and compared to the experimental results. The calculated values from the Morrow’s model obtained for each sample was plotted versus the experimental ones in figure 4b. In this figure, perfect correlation would be represented by data points lying on the solid diagonal line, and the dashed lines on either side of the diagonal represent error bands of a factor of $10^{34}$. Figure 4 shows that the model is able predict successfully the load recovery cycle behavior of PCL, PCL + fibrin and PCL cell seeded under free-swelling and dynamical loading. It was previously observed that the mechanical hysteresis of PCL scaffolds and its fatigue behavior is affected by the presence of water inside of porous structure, the main contribution of material fatigue behavior being given by aqueous media that acts as plasticizer and promotes a uniform distribution of the applied stress along the sample and not only in the trabeculae of PCL. Moreover, the incorporation of fibrin without cells or even PVA with different freeze/thawing cycles inside of polymer porous does not influence the fatigue material performance as much as the cell culture in different conditions does.

Figure 4b shows that the mechanical stability of PCL scaffolds after cell seeding was increased up to 600 cycles, which is higher than the ones observed for the PCL, PCL + fibrin and PCL filled with PVA. This result indicates that the material behavior is influenced by the presence of the ECM inside the porous scaffold and the mechanical behavior depends on factors related to the matrix generated by cells and not only on polymer elasticity and water homogenous distribution.

Failure can come from different reasons, such as physical and mechanical gaps between the scaffold and the matrix. The fatigue analysis was performed without cell fixation, in order to avoid chemical modification of the ECM and consequent artifacts on the mechanical measurements. However, these are performed in a harsh hypotonic environment, without proper nutrients and sterility, thus cells can die in the process. Although cells by themselves should not contribute to mechanical resistance of the construct, it is not known whether cell death can be affecting the ECM at the end. This problem can be addressed by evaluating matrix composition before and after the mechanical measurements.
**Quantitative real-time PCR**

**Figure 5**

The expression of collagen type I strongly decrease after 14 and 28 days of cell culture, indicating a reduction on fibrous-like matrix component. According to statistical analysis, changes in collagen I are significative with time, but changes in collagen type II are not, which remain similar, with a slight decrease. The ratio of expression between collagen type II and type I is subsequently increased with time, although at the end of the experiments there is still more relative expression of collagen type I than type II (ratio col II/col I lower than 1). No differences are observed between samples under mechanical stimulus and under free-swelling conditions.

KUM5 cells are precursors from mesenchymal origin that express collagen type II even without induction of differentiation. In our case, expression of collagen type II did not increase with time (a slight reduction is actually observed), suggesting that there is no induction by the specific medium nor the mechanical loads of chondrogenic trait. Nevertheless, there is a trend for reduction of collagen type I, indicating a decrease of that fibrocartilage component. This result, combined with the fact that no other matrix components and no other quantitative analysis for matrix deposition were performed, does not allow concluding that a chondrogenic matrix was obtained. An accurate analysis of fibrin degradation was not carried out, but most of the fibrin should have disappeared after 14 days. The presence of fibrin and its degradation rate can have an effect in the type of matrix that is produced. Although it can be thought that a culture without fibrin could have provided insight as a negative control, the size of the pores is bigger than the diameter of cells, and the surfaces of a scaffold of these characteristics, without a pericellular matrix, could act as a 2D surface, introducing geometric variables that would hinder comparisons. It is important to remark that fibrin, in absence of mechanical stimuli, induces more fibrous tissues in mesenchymal cells and favor, for example, myogenesis over chondrogenesis. It is possible that the initial two weeks of cell culture in the fibrin matrix without loading hinder production of collagen II, but the medium compensates some effects as the collagen type I expression is reduced. The lack of response of cells to mechanical loading
could be also related with fibrin, as it has been found that cyclic loading in fibrin hydrogels provokes mesenchymal stem cells to keep undifferentiated traits at the initial stages\textsuperscript{13}. If not all fibrin has been degraded when loading starts to be applied, or if the substitute matrix is of fibrous nature, it could be contributing to the absence of differences with dynamic loading during cell culture. This effect can be also caused by the scaffold permanent deformation.

**Discussion**

The produced matrix after cell culture with or without dynamic compression has a clear effect in modifying the fatigue properties of the constructs. Above all, presence of matrix results in different fitting values and better correlation to Morrow’s model than any PCL scaffold without cells. No other elements are present to produce this differences, because cell contribution to mechanical properties is negligible \textsuperscript{36}. When compared with a scaffold with the pores filled with a PVA gel (6 cycles), if the elastic modulus is the only parameter observed, samples with cells would be categorized as with the same mechanical properties than the latter.

**Conclusions**

Therefore, a main conclusion and consideration can be taken, and it is valid even with the matrix conditions: Elastic modulus cannot be the only descriptor necessary to characterize mechanical functionality of *in vitro* constructs with cells. It remains unclear whether the resistance to fatigue would improve if a matrix with more hyaline cartilage traits would have been achieved. Nevertheless, the behavior of the constructs with the extracellular matrix produced in this study is different than that of either scaffolds without cells and those filled with hardened PVA, thus it is reasonable to think that with a stronger chondrogenic induction of the cells the response to fatigue would be still improved. Therefore, fatigue tests appear as an important tool to completely assess the mechanical performance of scaffolds for implantation in cartilage.
Acknowledgements

We want to acknowledge Dr. João Pedro Silva and Dr. Leon Kluskens, from Departament of Biologic Engineering, University of Minho, for the help provided to work in the department, as well to Sylvie Ribeiro from the Department of Physics of the same university. This work is funded by FEDER funds through the "Programa Operacional Fatores de Competitividade – COMPETE" and by national funds arranged by FCT- Fundação para a Ciência e a Tecnologia, project reference PEST-C/FIS/UI607/2014. The authors also thank funding from Matepro – Optimizing Materials and Processes”, ref. NORTE-07-0124-FEDER-000037”, co-funded by the “Programa Operacional Regional do Norte” (ON.2 – O Novo Norte), under the “Quadro de Referência Estratégico Nacional” (QREN), through the “Fundo Europeu de Desenvolvimento Regional” (FEDER). The authors also thank support from the COST Action MP1206 “Electrospun Nano-fibres for bio inspired composite materials and innovative industrial applications” and MP1301 “New Generation Biomimetic and Customized Implants for Bone Engineering”. JAP, VS, CR and VC thank the FCT for the SFRH/BD/64586/2009 and SFRH/BPD/63148/2009, SFRH/BPD/90870/2012, and SFRH/BPD/97739/2013 grants, respectively. JLGR acknowledges the support of Spanish Ministry of Economy and Competitiveness (MINECO) through the project MAT2013-46467-C4-1-R (including the FEDER financial support). CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

References


**Figure Captions**

**Figure 1** – PCL microstructure: a) pristine scaffold, b) PCL+Fibrin c) PCL without fibrin seeded with KUM5 after 1 day of cell culture, d) KUM5 encapsulated in fibrin inside the PCL scaffold, after 1 day of cell culture. The big arrows indicate the location of cells and the smaller arrows point some of the protrusions of cells adhering to the pore walls.
Figure 2 – DNA content in full scaffolds during 21 day culture period with and without fibrin. Statistical differences are marked with asterisks.
Figure 3 – Mechanical hysteresis loops after cell culture on PCL+Fibrin scaffolds for a) free-swelling and b) dynamic cell culture in a bioreactor; c) Elastic moduli obtained for the first cycle for the different PCL samples and d) average maximum tensile stress as a function of the number of cycles.
Figure 4 – a) Relationship between the overall equivalent behavior similar to plastic strain energy density and number of load recovery cycles for the different PCL samples after different cell culture conditions, b) Comparison of experimental and predicted fatigue behaviors, calculated according to Morrow’s model.
Figure 5  - Folding changes (initial value of 2) with respect to β-actin housekeeping gene for a) collagen type I and b) collagen type II. c) Ratio of expression between collagen type I and type II. Statistically significant differences are marked with an asterisk.
### Table Captions

**Table 1** – Sequence of primers for target genes

<table>
<thead>
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<th>Gene</th>
<th>Forward primer 5’-3’</th>
<th>Reverse primer 5’-3’</th>
<th>Amplicon size (bp)</th>
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<td>CAATAGTGATGACCTGGCGTG</td>
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<td>Collagen type I</td>
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<td>Collagen type II</td>
<td>AGAACAGCATCGCCTACCTG</td>
<td>CTTGCCACTTACCAGTGT</td>
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**Table 2** – Fitting results after Morrow’s model (equation 2) for the different PCL scaffolds and after the different cell culture conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>m</th>
<th>C</th>
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<tbody>
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<td>PCL</td>
<td>0.24 ± 0.05</td>
<td>2500 ± 600</td>
</tr>
<tr>
<td>PCL + Fibrin</td>
<td>0.27 ± 0.04</td>
<td>2100 ± 660</td>
</tr>
<tr>
<td>PCL+PVA 6 cycles</td>
<td>0.16 ± 0.04</td>
<td>1865 ± 170</td>
</tr>
<tr>
<td>Free-swelling</td>
<td>0.22 ± 0.02</td>
<td>1445 ± 373</td>
</tr>
<tr>
<td>Loaded in bioreactor</td>
<td>0.20 ± 0.02</td>
<td>1171 ± 314</td>
</tr>
</tbody>
</table>