Culture and differentiation of human bone marrow-derived mesenchymal stem cells in poly (L-lactic acid) scaffolds

e-Poster: P155

Congress: ICRS 2009

Type: Electronic Poster

Topic: Basic Science / Scaffolds

Authors: I. Izal Azcárate¹, P. Ripalda Cemborán¹, P. Aranda¹, G. Mora¹, R. Escribano¹, H. Deplaine², J.L. Gómez-Ribelles², G. Gallego Ferrer², V. Acosta³, I. Ochoa³, J.M. García-Aznar³, M. Doblare³, F. Prosper¹; ¹Pamplona/ES, ²Valencia/ES, ³Zaragoza/ES

Keywords: Tissue engineering, Scaffolds, Stem cells
1. Abstract

Introduction: The lack of inflammatory response and the feasibility for controlling mechanical, morphological and degradation properties are attractive features of poly (L-lactic acid) (PLLA) for cartilage repair. Our aim was to determine the biocompatibility between Mesenchymal Stromal Cells (MSC) and PLLA and their effect on the mechanical properties of the scaffolds.

Methods and Materials: MSC were cultured in PLLA films to study adherence and proliferation. We loaded $10\times10^6$ and $2\times10^6$ MSCs into PLLA scaffolds (1 and 6 mm in thickness), either by injection or aspiration. Scaffolds were maintained in expansion or differentiation media for 21 days. Seeding efficiency and cell distribution and viability were analyzed after the seeding while chondrocyte differentiation was assessed at 21 days.

Results: Results showed an adhesion of MSC to PLLA films of $27.0\pm0.15$ and $37.7\pm8.7\%$ of seeded cells at 1 and 4 hours. Proliferation was detected, but ceases at day 10. An efficiency of $80.8\pm4.1$ and $71.5\pm9.4\%$ for 1 and 6 mm scaffolds was detected after the seeding. Retaining of cells were more efficient in the 6 mm scaffolds, where cells were poorly spread. Nevertheless, cells homogeneously saturated 1 mm scaffolds. Final results show the production of matrix composed by collagens I, and X, that produced a 2.6-fold increase in the Young modulus.

Conclusions: We have developed biocompatible PLLA scaffolds that can be efficiently loaded with MSCs. We also show that differentiation of MSCs can be successful in PLLA and that it modify the mechanical properties of the PLLA itself.

2. Purpose

The aim of this work is to study the chondrogenic differentiation potential of human bone marrow-derived mesenchymal stem cells cultured in PLLA scaffolds and the influence of the seeding technique and scaffold architecture.

3. Methods and Materials

Human bone marrow-derived MSC were first assayed for their capacity to differentiate to adipose, bone and cartilaginous tissue. Two sizes of PLLA derived scaffolds were used, both 6 mm in diameter and 1 and 6 mm in thickness. 6 mm thick scaffolds were seeded by injection with 2 millions MSCs, while 1 mm scaffolds were seeded aspirating 10 millions MSC through the scaffold. Seeding efficiency were measured by quantification of DNA, and distribution and density by fluorescence immunostaining of beta-actin. Retention of cells inside the scaffold at 21 days was also studied. Differentiation of MSC inside the scaffolds was performed by culturing them suspended in chondrogenic medium (CM) containing TGF-beta3 during 21 days. Matrix production was assessed by Massons tricrome staining and fluorescence immunostaining of aggrecan and collagens type I, II and X. Finally the mechanical properties of the differentiated scaffolds (Young modulus) was determined performing unconfined compression tests.

4. Results

1. Differentiation potential of human MSC (figure 1).
MSCs showed a potentiality to differentiate into adipose and bone tissue in monolayer, and to cartilage in micromass structure as it is shown in the figure.

2. Monolayer culture of human MSC on PLLA (figure 2)
Results show that $27.0\pm0.15\%$ of cells seeded anchored to the surface of the material after 1 hour.
When allowed for 4 hours, adherence reached a 37.71±8.7 %. Cells bound to PLLA did proliferate as it is shown in the graph. Cell proliferation begins on day 3, peaks at day 10 and finally decreases again from day 10 to day 14, without reaching a confluent state in the well (data not shown).

3. Three dimensional culture of MSC in PLLA scaffolds (figure 3)
Both seeding methods used resulted to be highly effective (80.6±4.1 and 71.5±9.4 % of cells for 6 and 1 mm thickness scaffolds respectively), but produced a considerably different distribution and density of cells. In 6 mm thick scaffolds, distribution of cells were highly restricted to the center of the scaffold (in the site of injection). On the other hand cells tend to spread over all the volume of 1 mm scaffolds. According to cell density, the poor distribution in 6 mm scaffolds combines with a high density, while a better distribution in 1 mm scaffolds, with a lower density. During a 21 days experiment, the quantity of DNA remains stable in 6 mm thick scaffolds but showed a progressive decrease in the 1 mm scaffolds.

4. Differentiation of MSC in PLLA scaffolds (figure 4)
When scaffolds were cultivated in the presence of CM, cells appeared surrounded by extracellular fibers that were positively stained using massons trichrome. The chemical nature of these fibers was studied and resulted in a predominant immunostaining for collagen type X in both 1 and 6 mm thick scaffolds. Aggrecan staining was also positive for both sizes, but showed a more intense pattern in 6 mm scaffolds. To a lesser extent, matrix fibers were also composed by collagen type I. Finally, the mechanical properties (Young modulus) were calculated. During the culture in expansion medium, the modulus significantly decreased only for 6 mm scaffolds, but increased with chondrogenic medium for both sizes.
Figure 2. Biocompatibility of PLLA to human MSCs. A total of 10,000 cells were allowed to adhere to PLLA films in 96 well plates during 1 and 4 hours. Average of the percentage of cells adhered (n=3) was calculated using MTS staining and represented in (A). The same number of cells were seeded and allowed to proliferate in the films cultivated in expansion medium. The average number (n=3) of viable cells, estimated by MTS, at different time points shows the pattern of proliferation of MSCs in PLLA (B). Error bars: standard deviation.
Figure 3. Three-dimensional culture of human MSCs in PLLA scaffolds. Two different protocols were used, injecting 2 millions of MSCs in 6 mm thick scaffolds and aspirating 10 millions in 1 mm thick scaffolds as it is indicated in the text. Images show the distribution of cells inside the scaffold for 6 (A) and 1 mm (B) scaffolds. (C) Seeding efficiency was calculated as the average of the quantity of DNA in homogeneized scaffolds (n=3) referred to the quantity in 2 or 10 millions of MSCs (for 6 and 1 mm scaffolds respectively). (D) The three dimensional distribution of MSCs inside scaffolds was studied calculating the average (n=3) of the the area occupied by MSCs in different sections of the scaffold. (E) Survival of MSCs in PLLA scaffolds was determined measuring the DNA content in a 21 days experiment. Average for every time point (n=3) is represented and referred to day 0. Error bars: standard deviation.
Figure 4. Chondrogenic differentiation of MSCs in PLLA scaffolds. (A) Masson’s trichrome staining of sections from 6 and 1 mm thick scaffolds showing the presence of matrix fibers. (B) Unconfined compression tests were used to determine the mechanical resistance of control and differentiated scaffolds compared. The average (n=5) is represented, compared with the same measurement in unseeded scaffolds (labeled as PLLA). (C) Immunofluorescence analysis of sections from 6 and 1 mm thick scaffolds used for the detection of Aggrecan, and Collagens type I, II and X.

5. Conclusions

1. Human bone marrow derived mesenchymal stem cells (MSC) have the potential to differentiate to chondrocyte phenotype when cultured in PLLA derived scaffolds as it is shown by the production of extracellular matrix (ECM). This matrix significantly improves the mechanical properties of the construct.

2. ECM produced by MSC in PLLA scaffolds differentiated suspended in chondrogenic medium is composed mainly by aggrecan and collagens type I and X. We suggest the lack of mechanical stimuli as the cause of the poor content in type II collagen.

3. The architecture of the scaffold and the seeding technique are issues that strongly influence the final construct, as it is shown by a higher production of aggrecan in 6 mm scaffolds (with a higher density of cells in the site of injection) and by the different mechanical behaviour observed in scaffolds used in the study.

6. References


5. von der Mark, K., Gauss, V., von der Mark, H., and Muller, P. (1977) Relationship between cell shape and type of collagen synthesized as chondrocytes lose their cartilage phenotype in culture. Nature 267, 531-532


7. Mediafiles

Figure 1

Assessment of the differentiation potential of bone marrow-derived human MSCs. Cells were pelleted and cultured in micromass spheres using CM to assess chondrogenic potential. Photomicrography shows staining using toluidine blue of a representative sphere after 21 days in culture (A). Alternatively, MSCs were cultured in monolayer in osteogenic and adipogenic medium during 21 days. Osteogenic differentiation was assessed using Alizarin Red and Alkaline Phosphatase staining (B), while adipogenic differentiation was shown using oil red staining (C).

Figure 2

Biocompatibility of PLLA to human MSCs. A total of 10,000 cells were allowed to adhere to PLLA films in 96 well plates during 1 and 4 hours. Average of the percentage of cells adhered (n=3) was calculated using MTS staining and represented in (A). The same number of cells were seeded and allowed to proliferate in the films cultivated in expansion medium. The average number (n=3) of viable cells, estimated by MTS, at different time points shows the pattern of proliferation of MSCs in PLLA (B). Error bars: standard deviation.
Figure 3. Three-dimensional culture of human MSCs in PLLA scaffolds. Two different protocols were used, injecting 2 millions of MSCs in 6 mm thick scaffolds and aspirating 10 millions in 1 mm thick scaffolds as it is indicated in the text. Images show the distribution of cells inside the scaffold for 6 (A) and 1 mm (B) scaffolds. (C) Seeding efficiency was calculated as the average of the quantity of DNA in homogenized scaffolds (n=3) referred to the quantity in 2 or 10 millions of MSCs (for 6 and 1 mm scaffolds respectively). (D) The three dimensional distribution of MSCs inside scaffolds was studied calculating the average (n=3) of the area occupied by MSCs in different sections of the scaffold. (E) Survival of MSCs in PLLA scaffolds was determined measuring the DNA content in a 21 days experiment. Average for every time point (n=3) is represented and referred to day 0. Error bars: standard deviation.
Figure 4. Chondrogecnic differentiation of MSCs in PLLA scaffolds. (A) Masson’s trichrome staining of sections from 6 and 1 mm thick scaffolds showing the presence of matrix fibers. (B) Unconfined compression tests were used to determine the mechanical resistance of control and differentiated scaffolds compared. The average (n=5) is represented, compared with the same measurement in unseeded scaffolds (labeled as PLLA). (C) Immunofluorescence analysis of sections from 6 and 1 mm thick scaffolds used for the detection of Aggrecan, and Collagens type I, II and X.