SHORT COMMUNICATION

O-16. OSTEOGENIC DIFFERENTIATION OF DENTAL PULP STEM CELLS ON POROUS SILICON LOADED WITH β-GLYCEROPHOSPHATE

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Porous Silicon, Dental Pulp Stem Cell, Differentiation, Bioresorbable

Introduction
Several studies have been carried out to verify if stem cells could become a source of stable differentiate cells capable of inducing tissue formation. Among these, mineralization of hard tissues is of great importance. Stem cells for hard tissue formation has thus considerably increased attention of researchers as these cells can be a possible, fascinating source of stable differentiated cells, capable of inducing bone formation and control hydroxyapatite crystal growth.
Human dental pulp stem cells (DPSCs) are able to produce, in vitro, calcified nodules and to form, after transplantation in vivo, a mineralized tissue. Supporting scaffolds for bone tissue engineering are often required to lead cell implantation, growth and differentiation (1).
Porous silicon (pSi) is a promising biomaterial that is non-toxic and biodegradable. Surface modifications offer control over the degradation rate of pSi, and it has an ability to degrade, in aqueous solutions, into non-toxic silicic acid (2).
In this work we have challenged oxidized porous silicon wafers stem cells from human dental pulps (DPSCs), to follow their osteogenic differentiation and thus evaluate their clinical use capability. DPSCs were challenged porous silicon, either with osteogenic induction or usual culture medium. During the cultures, cells were examined for histology, and enzymatic activity. Porous silicon gives interesting properties suggesting that it is a promising alternative for clinical use in bone regeneration.

Materials and Methods
Human dental pulp cells were collected from teeth of healthy adults following an already described protocol (1). These cells were incubated in DMEM-10%FBS at 37°C in 5% CO2 atmosphere in a humidified incubator and the medium was changed twice a week.
Cells were cultured for 10 days until they form sufficient number of colonies. They were analyzed by flow cytometry using mouse anti-human CD90-APC, CD146-FITC, CD117-APC and CD45-PerCP. Cells viability was assessed using LIVE/DEAD® Fixable Dead Stain Kit (Invitrogen). These cells were then incubated in DMEM on pSi samples with or without osteoinduction.
P-type silicon wafers (3–6 cm resistivity) were etched in a custom-made Teflon cell at a constant current density of 300 mA/cm2 for 2 min, in a 25% fluorhydric acid (HF) solution in ethanol, and oxidized at 800°C. They were then either loaded with β-glycerophosphate (for osteogenic differentiation) or used directly for cell culture (control group).
Cells were controlled for pluripotency with in vitro osteogenic, adipogenic and chondrogenic differentiation following a protocol previously described (3). Control cultures were maintained without differentiation medium.
To determine cell morphology and density on the surface, cells were stained with fluorescein diacetate (FDA) and 4',6-diamidino-2-phenylindole (DAPI), and observed under fluorescence microscopy. Cell proliferation was measured via quantification of acid phosphatase activity for both living and proliferating cells.

After 21 days, osteogenic, adipogenic and chondrogenic differentiation were assessed by histological staining with Alizarin red, Red Oil, and Alcian blue respectively. Osteogenic differentiation on pSi was followed by alkaline phosphatase (ALP) activity, determined by the hydrolysis of p-nitrophenyl phosphate at pH 10.4. ALP is a widely used marker, mainly expressed in differentiated cells producing mineralized matrix (4).

Results

Immunofluorescence analysis revealed that DPSCs cultures were positive for CD146 (74.5%), CD117 (44.2%) and CD90 (99.4%) (Fig. 1). All cultures were negative for the leucocyte precursor marker CD45. All cells were alive during flow cytometry analysis (Fig.2).
Multilineage differentiation of these DPSCs was enlightened with Alizarin red, red oil and Alcian blue staining, clearly showing calcification areas, lipid droplets, and proteoglycan synthesis, when cells were cultured respectively in osteogenic, adipogenic, or chondrogenic medium.
As early as one week after induction of osteogenic differentiation on pSi, cells started to express alkaline phosphatase, whereas no such activity was detectable without osteogenic induction. ALP expression remained at high levels after 3 weeks in culture. In uninduced cultures, ALP activity was much lower compared to the induced cultures.
Fluorescent microscopy with FDA and DAPI staining revealed that cells adhered significantly better on culture plate but presented the same morphology on pSi as on culture plate (Fig. 3).

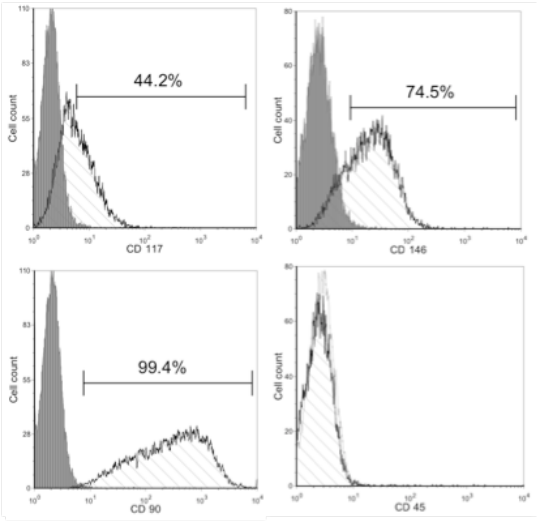

Figure 1: Flow cytometry analysis after 10 days of expansion. Gray area: isotype control; black line: marker of interest.

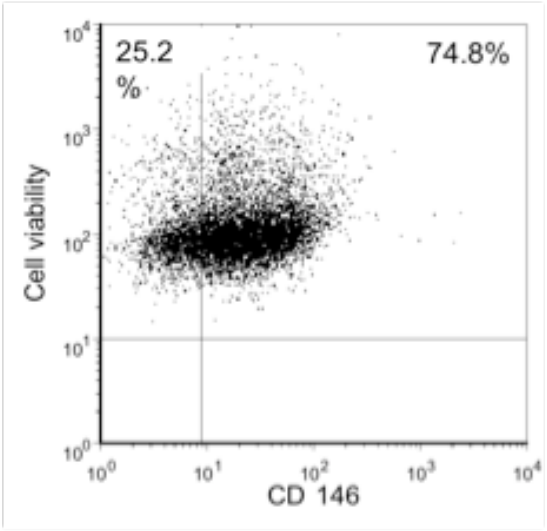

Figure 2: cell viability vs. antibody marker. All cells appeared to be vital.

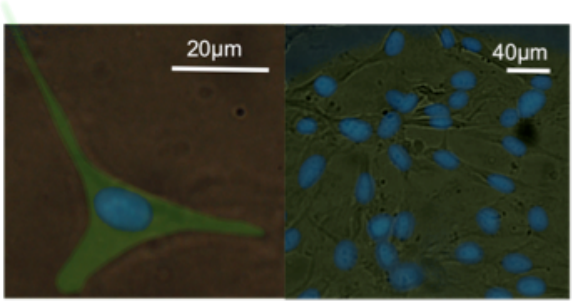

Figure 3: Fluorescent microscopy, FDA and DAPI staining, magnificence x400 and x200.

Discussion

DPSCs seeded on pSi presented the same characteristics as those on culture plates although their initial adhesion was lower. Osteoinduction on pSi leaded to differentiation into cells expressing ALP, meaning that these cells were able to produce a mineralized matrix. Thus, porous silicon can be a suitable scaffold for bone tissue engineering, allowing DPSCs growth and differentiation. This porous material can be loaded with β-glycerophosphate, which is progressively released during pSi resorption. Cells can thus use the released β-glycerophosphate for mineralized matrix building.
Porous silicon has been extensively studied these last years for cell growth and biocompatibility. As its porous structure allows different molecules loading, other ways of differentia-

tion could be explored for tissue engineering.

References

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