RESPONSE OF ASC CELLS TO PRESSURE STIMULATION ON OXYGEN TERMINATED NANOCRYSTALLINE DIAMOND SURFACE

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Introduction

One of the methods utilized in the treatment of irreparable damage to bone is bone replacement by synthetic implant. It is usually made from metal, ceramic or combination thereof. After implantation into the patient together materials and living tissues interact [1]. The reaction is dependent on the physicochemical properties of the material, which may cause various cell responses, thus affecting the adhesion of cells to the implant [2]. To eliminate problems in the interaction of the implant with the surrounding tissue implant surface is modified by applying different surface layers. Appropriate modification of the surface can modify the functional interface bone – implant – it means increasing of osteointegration, reducing the healing time and allowing for early loading of the implant [3]. The starting point of the above disadvantages is covering of the implant surface by autologous cells of the patient. Many studies showed that static cultivation supports insufficiently osteoblast differentiation of stem cells [4]. Dynamic cultivation with defined stimulation can improve this process and creates more *in-vivo* like conditions.

Materials and Methods

The aim of this study was to achieve response of adipose stem cells (ASC) on nanocrystaline diamond surface NCD under pressure stimulation in vitro. A custom-built bioreactor was created. This system consists of the specially designed cultivation chamber and pressure generating linear pump. The cultivation chamber allows fixing NCD substrate and creates reservoir for culture medium. The cells are seeded there over sterile septum. The overall construction was optimized for time-lapse microscopic imaging. Computer controlled custom-built linear pump with pressure sensing is used as pressure generator. This solution allows setting pressure range up to 700 mmHg and frequency up to 2 Hz.

The substrate both for dynamic and static cultivation was tissue treated glass (dimensions 24x24 mm, 0.15 mm thickness) with deposited a thin layer of NCD. The thickness of NCD was approximately 200 nm (depending on the length of the deposition). This layer was in the last step of deposition terminated with oxygen, resulting in a high surface wettability.

The substrates were seeded with density of 70 000 cells/cm^2. This density was set to get nearly confluent coating. After 1 hour a pulsatile cyclic strain of 50/100 mmHg, at a frequency of 0.2 Hz was set for 71 hours. The static control was left without stimulation. The fresh DMEM culture medium supplemented with 10% FBS, 50 ug/ml ascorbic acid, 10 mM B-glycerophosphate, 10 nM dexamethasone was used. After 72 hours, the substrates were removed and the cells were fixed and fluorescently stained for further analysis.

Results and Discussion

There was significant difference in morphology between dynamic and static cultivated cells observed by light microscopy. Analysis of alkaline phosphatase, collagen-1 and osteopontin using fluorescent staining observed that stimulated cells express scientifically more genes than static cultured cells. These 72hours experiments were aimed at verifying the hypothesis of advantages of dynamic stimulation and testing of stimulating system. In the future experiments with longer duration are supposed to achieve differentiation of stem cells into osteoblasts.

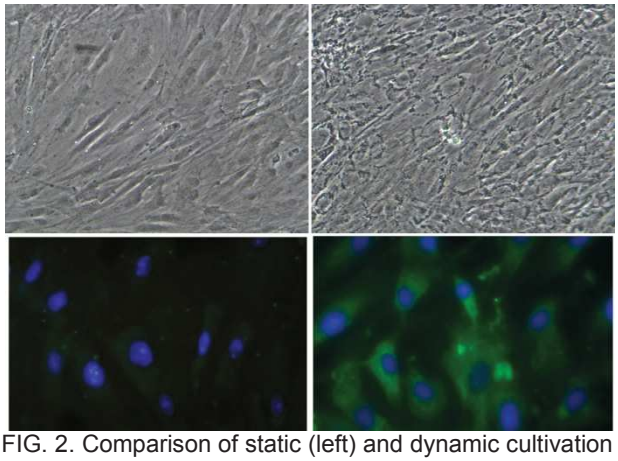

FIG. 2. Comparison of static (left) and dynamic cultivation (right), in phase contrast microscopy (top) and collagen-1 fluorescence staining (bottom).

Conclusions

Dynamic cultivation with pressure stimulation of ASC cells on O_2-terminated nanocrystaline diamond substrates has influence of cell proliferation and supports production of early markers of osteogenic differentiation in contrast to static cultivation.

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