

## HUMAN OSTEOGENIC CELLS RESPOND TO ION IRRADIATED POLY- $\epsilon$ -CAPROLACTONE-BASED SURFACE

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**INTRODUCTION:** A variety of natural and synthetic polymers are under development for filling bone defects. A step to be taken prior to clinical application of engineered materials is the assessment of the osteoconductive potential *in vitro* and *in vivo*. Among compatible polymers suggested for bone engineering poly- $\epsilon$ -caprolactone (PCL), as well as some co-polymers, is receiving considerable attention due to its excellent physical properties, compatibility with tissues, and controlled degradation depending on porosity or co-polymerization.

**METHODS:** PCL films were deposited on p-doped silicon wafers and some samples were irradiated with low-energy He<sup>+</sup> ions. The resulting surfaces were characterized, including surface wettability and roughness by surface free energy measurements and Atomic Force Microscopy, respectively. Human bone marrow was obtained from patients undergoing total hip replacement and isolated by gradient centrifugation. Marrow stromal cells (MSC) were then obtained by adherence on TCPS and expanded in osteoblast-inducing medium. Cells were seeded onto 0.5cm<sup>2</sup> squared PCL samples, with and without irradiation (PCL<sup>ut</sup> and PCL<sup>irr</sup>), and analyzed at different time endpoints. Alamar, ALP, collagen, mineralization, as well as fluorescence microscopy and SEM, were employed for detection of bone marrow cell response to bare and irradiated PCL.

**RESULTS:** At the 1st time endpoint, i.e. 4h, different assays were run to look for early interactions of MSC with the surfaces.

By fluorescence microscopy a higher number of nuclei and an evident spreading of MSC onto PCL<sup>irr</sup> compared to PCL<sup>ut</sup> were observed.

Using an image analysis software the views were quantified (table I).

Table I. Image analysis of MSC (number and spreading) on PCL surfaces at 4 h from seeding.

sample	n° of nuclei	spreading (area fraction)	area ( $\mu\text{m}^2$ )	measured area ( $\mu\text{m}^2$ )
PCL <sup>ut</sup>	8 $\pm$ 3	~ 0	618.52	6.8 x 10 <sup>5</sup>
PCL <sup>irr</sup>	74 $\pm$ 9	0.101	68,481.6	6:8 x 10 <sup>5</sup>

Cell viability assayed at different time endpoints using Alamar test showed a higher number of MSC on PCL<sup>irr</sup> (fig. 1).

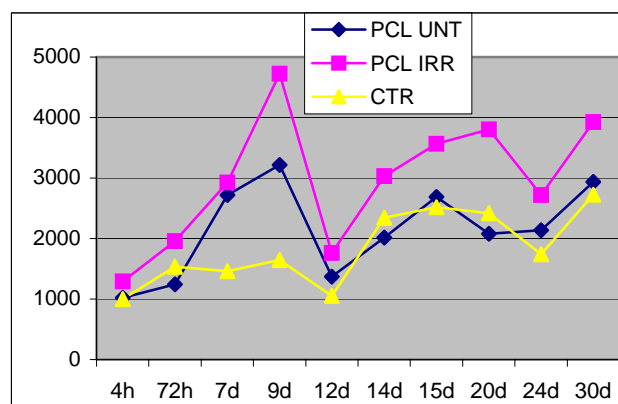


Fig.1 Viability assay of MSC grown on PCL surfaces and TCPS (Alamar blue, RFU units)

At 2 weeks alkaline phosphatase (ALP) was 9.32 mM for MSC on PCL<sup>irr</sup> compared to 5.69 on PCL<sup>ut</sup> (control MSC: 14.94). Collagen assay showed that the differentiation of MSC to ECM-forming cells, i.e. osteoblastic cells, was enhanced starting at 2 weeks for PCL<sup>irr</sup>.

At 4 weeks the MSC were multilayered on PCL surfaces (fig. 2) and start to detach from TCPS.

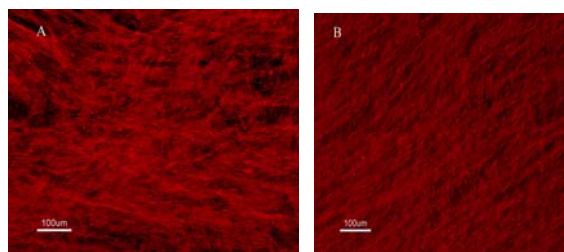


Fig. 2. MSC confluency on PCL<sup>ut</sup> (A) and PCL<sup>irr</sup> (B) at 1 month (phalloidin-TRITC)

**DISCUSSION & CONCLUSIONS:** From the results of biochemical assays, the general trend of cellular activities toward bone deposition is easily recognized for control MSC, while MSC on PCL surfaces show some deviation. Proliferation of MSC on PCL<sup>irr</sup> is higher than onto PCL<sup>ut</sup>, and mineral formation also confirms a better behaviour of irradiated PCL surface vs untreated PCL. These *in vitro* results can be correlated to structural features, i.e. wettability and roughness of PCL.