3D Gene Activated PEG Matrices Designed to Direct Cell Migration
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Introduction
The realization of systems able to both direct cells migration and influence their fate represents a new approach for tissue regeneration. We investigated the potency of gene activated matrices (GAM) [1] and implemented the GAM strategy in order to achieve also a guided cell migration. The cells migrate into the matrix where they find the pDNA complexes bounded to the matrix, and are transfected. The transfected cells act as local in vivo bioreactors, secreting plasmid encoded proteins that augment tissue repair and regeneration. In order to experimentally validate this methodology, we realized a DNA bioactivated high porous PEG matrix by Polyethylenimine (PEI)/DNA complexes [2] immobilization in a PEG hydrogel with a designed inner structure [3]. The matrix was realised with a gradient of the adhesive peptide sequence RGD in order to direct cells motion.

Materials and Methods

Hydrogels preparation
To create porous hydrogels, we put 50% of home-made uncrosslinked gelatine micropartricles (d=150-300 μm) into gaskets. Then we poured a solution containing PEG diacrylate (PEGDA) and a UV light-sensitive radical around these beads and exposed to UV light for 3 min in order to polymerize the diacrylate. After polymerization, the gelatine beads were leached away from the hydrogels using water at 37°C over 24h.

To biologically functionalize these hydrogels for cells adhesion, RGD was conjugated to PEG monoacrylate and photopolymerized with PEGDA. To genetically functionalize these gels PEIpDNA complexes were immobilized into the hydrogels by covalently linking PEI to PEG before photopolymerization. In order to spatially design the distribution of the bioactive site (peptides and PEIpDNA complexes) into the hydrogels a gradient maker device was used upstream the mold.

Complexes formation
Plasmid DNA encoding for green fluorescent protein (GFP) was complexed with PEI at a nitrogen/phosphate ratio (N/P) of 5.

Cell culture and transfection
The efficiency of gene transfer by the DNA activated matrix was detected through fluorescence microscopy, evaluating the percentage of fluorescent cells (GFP expressing) on the total cell number.

3D cell migration
3D NIH3T3 cell migration in PEG-RGD matrices was monitored by time-lapse videomicroscopy.

Results
Experimental results show that we are able to produce a cell instructed hydrogel characterized by spatially predesigned inner structure in terms of: 1) porosity; 2) spatial gradients of RGD peptides; 3) biodistribution of PEIpDNA complexes.

Discussion and Conclusions
Gene activated matrices able to direct cell motion and transfect cells strategies, represent a powerful “tool” for tissue regeneration. The effectiveness for the target cells to find DNA drug and migrating within the matrix, here presented, is a valid extension of the tissue engineering concept and offers a wide range of new application to restore and regenerate damaged tissues. Furthermore our methodology allows a step forward producing gene activated matrices with high precision, and, above all, under very mild physical-chemical conditions.

References