Fluorescent quantification of osteogenesis of GFP-transgenic mouse calvarial osteoblasts on biomimetic coatings

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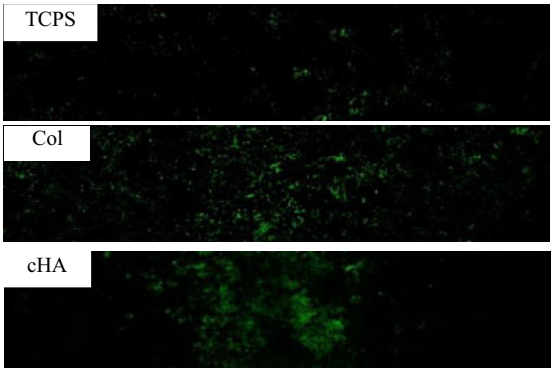
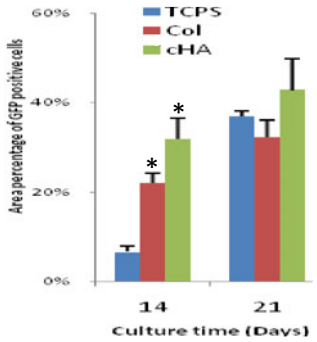
Statement of Purpose: Traditional assays for osteogenesis provide only average outcomes of cultures, but are not reflective of the heterogeneous differentiation or spatial arrangement of individual cells. In addition, termination of cultures is required so progression cannot be followed at specific locations. Genetically engineered cells that express green fluorescence protein (GFP) driven by cell type-specific promoters can provide such information. *Col1a1* promoter driven transgene pOBCol2.3GFP has been successfully used to identify stages of differentiation of mouse osteoblasts [Dacic et al., 2001; Wang et al., 005]. Here we apply this technology to tissue engineering and monitor spatial and temporal behaviors of cells on a biomimetic carbonated hydroxyapatite (cHA) and fibrillar collagen (Col). Additionally we report a method to quantify GFP expression. The generalized method could be used to study differentiation in any osteogenic tissue engineering application.

Methods: Primary osteoblast cells were harvested from the calvaria of 5-8 days old neonatal Col2.3GFPemd transgenic mice (mCOBs) [Dacic et al., 2001]. A cHA coating was applied to tissue culture polystyrene (TCPS) disks and its structure was confirmed by XRD, FTIR and EDX. A Col coating was applied to non-tissue culture treated plastic. TCPS was a control. Cells were plated at a density of 1.5 x 10^4 /cm^2 onto the three surfaces. The medium was changed on the second day and then every other day until day 7 after which a differentiation media was used and exchanged every other day for 21 days. A computerized stereo microscope (SteREO Lumar V12, Carl Zeiss) was used to visualize the culture expressing GFP. Series of 12x4 adjacent pictures (15% overlap) at 5x were concatenated into single images. Concatenated images from different time points were input into the ImageJ program (NIH) and adjusted to a common threshold to quantify the area and intensity of fluorescent expression.

Results: GFP expression at day 3 and 7 was not significant. At 14d a large number of cells began to express GFP as visualized on the concatenated images shown below. More GFP positive cells were observed on cHA and Col than on TCPS. GFP expression on cHA displayed large continuous regions. In contrast, GFP positive cells on Col or



TCPS did not form continuous regions, but were sparsely distributed. GFP expression increased up to day 21 on all surfaces. The percentage of the surface area covered by GFP positive cells at 14d and 21d is shown below. Approximately 4.3-fold and 2.7-fold more GFP positive area was observed on cHA and Col compared to TCPS, respectively, at 14d. The difference was not statistically significant at 21d ($p>0.05$). GFP fluorescence intensity was higher on cHA and Col than on TCPS at 14d ($p<.05$), but comparable at 21d.



Conclusions: GFP expression on three biomaterial surfaces was successfully quantified. Recognizing that Col2.3 GFP expression is strictly limited to mature osteoblasts, the higher level of expression on cHA and Col indicated earlier osteogenic differentiation on these two materials compared to TCPS. The GFP positive cells on cHA formed continuous regions compared to the sparely distribution arrangement on Col and TCPS.

References: Dacic S, et al. J Bone Miner Res. 2001; 16: 1228-1236. Wang YH, et al. J Bone Miner Res. 2005; 20: 5-14.