Using Molecular Beacon Probes to Image β1 Integrin Expression in Live Osteoblast-Like Cells

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Statement of Purpose: Molecular beacons are probes that can be delivered into live cells to allow for real-time imaging of mRNA. Molecular beacons have a hairpin structure, including a complementary sequence, allowing them to unwind when bound to the target mRNA sequence, resulting in a fluorescent signal upon excitation. A novel molecular beacon that is specific to the β1 integrin has been developed and used to image osteoblast-like MG63 cells in-vitro on glass and titanium surfaces. The titanium surfaces have been extensively characterized and are used clinically in implants[1]. Integrins form dimeric receptors that regulate a wide array of processes related to cell adhesion and osteoblast maturation. This technique provides a reliable tool to study changes in gene expression in individual cells at all stages of attachment and culture.

Methods: Six different molecular beacons targeting different locations on the β1 mRNA and a random sequence molecular beacon were designed, synthesized, and tested. For all imaging experiments, MG63 cells were plated at 5,000 cells/cm^2 on tissue culture treated glass, pre-treated titanium disks (PT, Ra<0.2 μm), or a sand-blasted acid etched titanium surface (SLA, Ra=3-4 μm). After 72 hours, molecular beacons (1μM) were delivered into live MG63 cells using reversible permeabilization with activated Streptolysin O (SLO, 0.1U/mL). Cell membranes were then allowed to reseal by aspirating the SLO solution and adding 250μL of the standard serum containing growth medium with 1μg/ml Hoechst33342 (Invitrogen) nuclear stain and incubating cells for 30 minutes at 37°C prior to fluorescent imaging.

Following identification of the beacon with the highest signal intensity compared to the random sequence, the beacons were delivered into MG63 cells and shRNA transfected MG63 cells to silence the β1 integrin, as previously described[1]. Silencing was verified using qPCR on all the surfaces at confluence with the cells plated at a density of 10,000 cells/cm^2. For imaging, 20 random fields were selected and the image stack was deconvolved using the DeltaVision™ software. The beacon signals of the cells were quantified by making a projection of the image stack, setting a threshold to remove the background, then summing the intensity in regions outlining each cell. The data from the qPCR and beacon quantification were analyzed using an un-paired, two-tailed t-test assuming unequal variance.

Results: The results from the qPCR showed a significant decrease in the integrin β1 expression of shRNA MG63 cells: 68.7% on glass, 65.4% on PT, 22.1% on SLA ($p<0.05$); as compared to the MG63 cells. There was also a significant difference between the SLA and both the PT and the glass surfaces in wild-type MG63 cells, but there were no differences among the shRNA MG63 cells ($p<0.05$). This suggests that the surface roughness affects integrin expression and that silencing the β1 integrin eliminates this surface effect. The image quantification from the molecular beacons showed the same significant decrease in signal intensity of the shRNA MG63 cells compared to the MG63 cells all surfaces: 65.0% on glass, 61.3% on PT, and 26.2% on SLA ($p<0.001$), (Figure 1). The molecular beacon signals were found diffusely throughout the cytoplasm on all surfaces (Figure 2). These results suggest that the molecular beacon developed is specific for the β1 subunit and that it can be used to image live cells on different titanium surfaces.

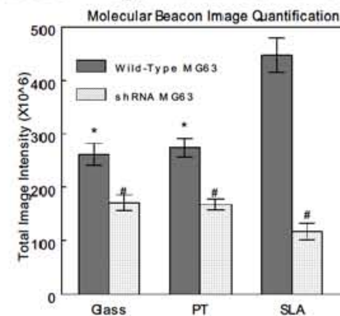

Figure 1: Comparison of molecular beacon image quantification on glass, PT, and SLA surfaces between MG63 and shRNA MG63 cells. All surfaces had a significant decrease in intensity of 65.0% on glass, 61.3% on PT, and 26.2% on SLA (#=P<0.001 wild-type vs. shRNA, *=P<0.05 SLA vs. glass and PT).

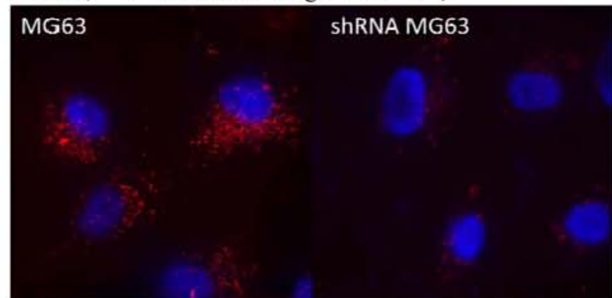

Figure 2: Representative image of molecular beacon in live cells on PT. The molecular beacon signals (red) are diffusely throughout the cytoplasm and not nuclear (blue).

Conclusions: These experiments suggest that the molecular beacon developed provides a reliable way to detect gene expression in live cells, and also provides information about gene localization. A limitation to traditional gene expression techniques is the requirement of having a large number of cells to extract enough RNA to perform the assays, making it impractical to be able to study cells before confluence. By studying a range of individual cells, it is possible to detect changes in gene expression before the cells are confluent. Future work for the molecular beacons would include imaging at early points of cellular attachment and continuous imaging of the same live cells.

Reference: 1. Wang, L. Biomaterials. 2006;27: 3716–3725.

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