In-Vitro and In-Vivo Studies of Osseointegration on Laser Micro-grooved/RGD Functionalized Ti 6-Al-4V Surfaces

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Statement of Purpose: Cell contact guidance on textured surfaces is shown to decrease the formation of scar tissue while promoting improved cell/surface integration to laser micro-grooved and RGD functionalized titanium surfaces. This paper presents the results of in-vitro and in-vivo studies of the effects of laser microgroove geometry and RGD coating on cell spreading, adhesion and osseointegration. The in-vitro studies suggest that laser microgrooves promote contact guidance and improved cell adhesion, which is quantified using a trypsin detachment method. The in-vivo studies show that the combination of RGD coating and laser micro-grooving results in the best combination of bone formation and reduced scar tissue formation.

Methods: A mill annealed Ti-6Al-4V billet was used in this study. Grooves with widths and depths of ~ 10-20 um, and spacing from 20 um to 60 um, were introduced onto the surfaces by laser processing, which was carried out at Spectra Physics Inc., Mountain View, CA, using a Spectra-Physics Navigator II YHP40 laser. The underlying microchemistry changes due to laser processing were examined by EDS. The multi-scale geometries/roughness on Ti-6Al-4V surfaces were characterized by profilometer, SEM, and AFM. To reveal the microstructure, the specimens were cross-sectioned, polished, and etched with Kroll's reagent, which is a solution of 100ml H_2O + 5 ml HNO_3 + 3 ml HF. SEM was used to observe the cross-section to check the heat affect zone (HAZ) and solidification cracking caused by laser processing. HOS cells were cultured on the laser-grooved surfaces for 15mins, 1hour, 4hours and 24 hours, followed by SEM observation, which indicates the initial cell spreading and alignment process on surfaces. Confocal microscopy was used to examine the distribution of focal adhesion points by staining vinculin during the whole process. Finally, in-vivo studies of osseintegration were carried out in Alexandria, Egypt, using a rabbit model. These employed laser microgrooved and RGD coated pins that were produced at Princeton University. The in-vivo studies were used to explore the extent and type of bone formation over a period of about 90 days.

Results/Discussion: Relatively straight and uniform micro-grooves were produced in Ti-6Al-4V. Unlike the micro-grooves currently produced using large area masking techniques and Excimer lasers, the micro-grooves produced in this study with DPSS UV lasers do not result in extensive heat affected zones or resolidification micro-cracking. The mechanism of HOS cells contact guidance on laser-grooved Ti-6Al-4V surfaces was also elucidated before identifying the groove and RGD coating conditions for in-vivo studies. The in-vivo studies revealed that combined RGD coating and laser micro-grooving results in accelerated bone formation and wound healing, improved osseointegration and reduced scar tissue formation in rabbit models.

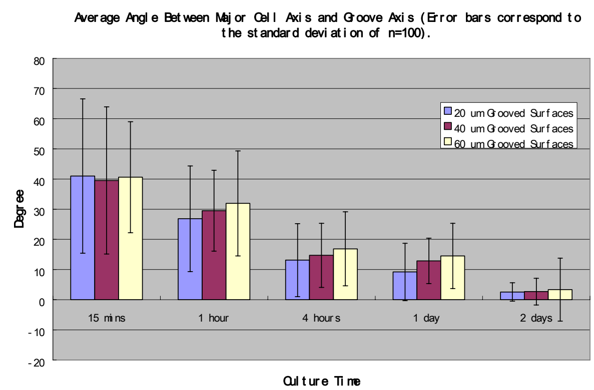

Figure 1. Cell orientation angles on laser grooved surfaces versus culture time

Conclusions:

1. Unlike the micro-grooves currently produced using large area masking techniques and Excimer lasers, the micro-grooves produced in this study with DPSS UV lasers do not result in extensive heat affected zones or resolidification micro-cracking.
2. Cell contact guidance was observed on laser grooved surfaces. For the range of micro-grooved geometries studied, micro-grooves with spacing of 20 um, provide the fastest speed of cell alignment to laser textured Ti-6Al-4V surfaces.
3. The combination of laser microgrooving and RGD coating results in accelerated wound healing, improved osseointegration and bone formation, and reduced scar tissue formation in rabbit models.