Effect of modified stainless steel 316L substrates on 3T3 fibroblast cell attachment

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Statement of Purpose: Cardiovascular diseases (CVD), especially coronary artery diseases are one of the leading causes of deaths in the United States. Even after angioplasty, there is a possibility of restenosis (reclosure of the artery) and thrombosis (blood clot formation). A stent, most commonly made of medical grade stainless steel (316L) or Nitinol is used to prevent the reclosure. While these devices are mostly successful, there are still problems that arise due to restenosis and thrombosis formation. To prevent biomaterial failure due to neoinitima and thrombosis formation after the insertion of stents, we have developed a novel method of formation of ordered, covalently bound self assembled monolayers (SAMs) on the oxide surface of stainless steel using two different acids - carboxylic acid and phosphonic acid with different tail functional groups of methyl, amino, hydroxyl and carboxylic acid to prevent the adhesion of platelets and cells and thus making the alloy biocompatible. Fibroblast cell attachment to these different terminated SAMs has been studied.

Methods: Self assembled monolayers of octadecyl phosphonic acid and carboxylic acids with different terminated groups were formed by solution deposition method on stainless steel 316L substrates. The films formed were analyzed by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy and tested for chemical and mechanical stability using sonication and adhesion tests. X ray photoelectron spectroscopy (XPS) was conducted on octadecylphosphonic and octadecylcarboxylic acid treated stainless steel to determine the chemical bonding of the acid to the substrate. Contact angle measurement was used to determine the wetting on the surface. Atomic force microscopy was used to image the surface to confirm coverage.3T3 fibroblast cells (10,000 cells/well) were seeded into wells containing the different acid modified 316L substrates to study the effect of terminal group on the number if cells attached. Live-dead assay and viability calculations after 1 day provided data about the number of cells attached to the modified substrates. Four different experiments were conducted and ANOVA used for statistical significance of the data.

Results / Discussion: The CH_2 asymmetric stretch value of <2918 cm^{-1} provided evidence for a well ordered all-trans conformation as observed by DRIFT spectroscopy. The SAMs were chemically and mechanically strongly bound to the substrate as confirmed by DRIFT spectroscopy after the rinse and adhesion tests. XPS analysis of the control stainless steel substrate after the polishing steps indicated that the surface composition, including the oxide content of the surface had not been significantly altered by the polishing step. Phosphonic acid was found to form bidentate binding with stainless steel while carboxylic acid formed predominantly bi-dentate even though mono-dentate bonding was also observed by DRIFT spectroscopy. Contact angle analysis showed that the methyl terminated SAMs had a value of 108° which is consistent with a hydrophobic surface while the hydrophilic terminated SAMs had contact angle values lower than control stainless steel substrate (45°). AFM of the modified surface showed complete coverage. The number of cells attached to the methyl terminated SAMs were found to be almost 80% less when compared to hydroxyl terminated, carboxylic acid terminated and amino terminated SAMs. ANOVA results showed a significant difference between all the modified substrates and the control sample except the amino terminated sample.

Conclusion: We have successfully formed ordered self assembled monolayers of phosphonic and carboxylic acids with different tail terminus on stainless steel substrates. Cell experiments with 3T3 fibroblast cells have shown that fewer cells attach to methyl terminated SAMs compared to the hydrophilic groups. We conclude from our experiments on 3T3 fibroblasts that octadecylphosphonic acid and stearic acid are better candidates than amine, hydroxyl and carboxylic acid to prevent cell attachment on stents.