Novel Biocompatible Adhesives from Plant Derived Monomers

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Statement of Purpose: Recent advances in genetic engineering, composite science, and natural fiber development offer opportunities for new polymer materials derived from renewable resources that are biocompatible and biodegradable. We have synthesized new high-performance, low-cost biocompatible materials from plant oils and their fatty acids for use as tissue scaffolds, wound healing dressings and load bearing implant materials. By selecting the fatty acid distribution function of plant oils via computer simulation and the molecular connectivity, we can control chemical functionalization and molecular architecture to produce

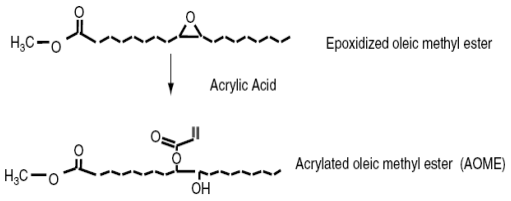

Figure: Structure of the plant oil derived monomer AOME.

linear, branched, or cross-linked polymers. The resulting polymers have a useful range of thermal and mechanical properties and exhibit adaptive surface energies suited to biological interactions. Here we report on initial biocompability and bioactivity assessments of these materials.

Methods: Acrylated oleic methyl ester (AOME) was synthesized via the methods discussed by Bunker et al.[1] A set of pressure sensitive adhesives were prepared by mini-emulsion polymerization according to Bunker et al.[2] Different types and ratios of co-monomer were used to generate adhesives with different mechanical and thermal properties and the compositions are listed. *Material for monomer synthesis:* Methyl oleate (99.9+% purity), formic acid (98% aqueous), hydrogen peroxide (30% aqueous), acrylic acid, and hydroquinone were used as received without further purification (Aldrich Chemical Co.). AMC-2, supplied by Aerojet chemicals, was used as received. *Material for polymerization:* EGDMA and MMA from Specialty Polymer, Inc. were freed from stabilizer by distilling using DHR-4 column (Scientific Polymer Products Inc.) Cobalt naphthenate (CoNap, Witco Corp.), Trigonox 239 (Akzo Nobel), sodium dodecyl sulfate (Aldrich Chemical Co.), Dicumyl peroxide (Aldrich), Styrene (Fisher), and 2,2-azobis(2-methybutyronitrile)(Vazo67, duPont) were used as received.

Results: *Cytotoxicity tests:* Cell viability on AOME/MMA adhesives was determined by performing an MTT-based *in vitro* toxicology assay (Sigma-Aldrich, St. Louis, MO). WS-1 Human dermal fibroblasts (ATCC, Manassas, VA) were used as the test cells. Test materials were placed in 6 well plates and seeded with approximately 800,000 cells in 2.5 ml of DMEM. Controls were created by seeding empty wells. The plates were incubated for 2 hours to assess cell attachment via optical microscopy. Samples were then incubated for a total of 24 hrs. After optical images were recorded at 24 hrs, the MTT assay was performed. All of the adhesives exhibited either stable or increasing cell number after 24 hours of incubation. Cells were monitored by optical microscopy to determine cell attachment rates and

Sample#	*% AOME*	*% MMA*	*% EGDMA*	*% Styrene*	*ACTA2 vs. Control*
1	100	0	0	0	4.98
2	91	0	0	9	2.29
3	95	5	0	0	8.79
4	80	20	0	0	4.75
5	79	20	1	0	4.61
6	79.3	20	0.7	0	7.67
7	59	40	1	0	5.71

phenotypes. All of the materials supported fibroblast attachment at 2 hours and spreading at 24 hours. Culture was maintained on the foam materials for 3 weeks. Fibroblasts continued to divide until the culture was stopped. Cells made attachments across the open pore structure and filled in open pores over time.

Gene Expression Analysis: We measured alpha smooth muscle actin mRNA expression in cells attached to the library of co-polymers listed. qPCR tests were performed in triplicate at the 8 hour time point (Applied Biosystems, Foster City, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPD) was used as the endogenous control. The $2^{-\Delta\Delta C}{}_{T}$ method of quantifying relative changes in gene expression[3] was applied to determine the mean relative fold changes between cells growing on tissue culture plastic and the test materials. All of the materials induced an upregulation of alpha smooth muscle actin (ACTA2), a marker for myofibroblast differentiation, in attached cells. This may indicate that that these materials are capable of inducing a wound healing response in these cells.

Conclusions: These initial results indicate that these adhesives offer a promising new class of biocompatible and bioactive materials. We demonstrated that AOME/MMA was supportive to cell attachment and proliferation. We also demonstrated differential gene expression of genes involved in wound healing between cells grown on the new materials and cells grown on control surfaces

References: 1. Bunker SP, Wool RP. Journal of Polymer Science Part a-Polymer Chemistry 2002;40(4):451-458. 2.Bunker S, et al. International Journal of Adhesion and Adhesives 2003;23(1):29-38. 3. Livak KJ, Schmittgen TD. Methods 2001;25(4):402-8.