Surface immobilization of model protein onto new photo cross-linkable materials

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Abstract:

For the effective screening of new photo cross-linkable materials for the fabrication of customized surface reactive scaffolds produced by photolithography, a selective and indicative method was needed that would allow for the identification of those polymers which have the best surface properties to be modified by means of covalent protein binding.

As a model protein, alkaline phosphatase (ALP) was chosen because a sensitive detection assay is available and the assay allows for distinction of enzymatically active protein from denatured molecules. Reaction conditions for covalent surface immobilization were optimized for buffer type, pH, temperature and time. Enzyme activity was determined by conversion of p-nitrophenolphosphate in p-nitrophenol.

Test surfaces were based on trimethylolpropane triacrylate (TTA) or adipic acid divinyl ester (AVE) discs. These monomers were photopolymerized into discs and 5% of a panel of reactive monomers (GMA, VEC, PCVC) were added to obtain reactive surfaces. All reactive groups have a documented affinity to free amine moieties in peptides and proteins. The modified polymers were tested in comparison with non-reactive TTA and AVE to determine the amount of adsorbed and covalently bound protein. The described method for protein detection was found to be very sensitive allowing for the detection of as little as 2 ng ALP. Surface modification was successful for a number of different materials. Statistically significantly ($p<0.01$) increased amounts of ALP as compared to the control surfaces (TTA and AVE) were found on TTA/GMA, AVE/VEC and AVE/PCVC discs.