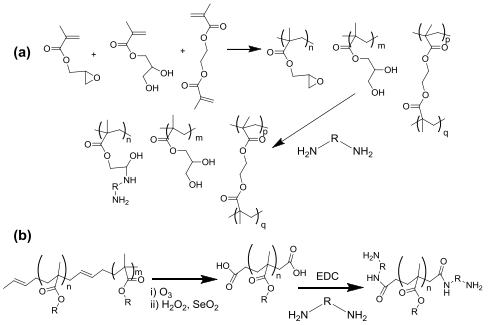
Cell Adhesion to Alkyl Aminated Hydrogels and Coatings

Stephen Rimmer,[1] Sheila MacNeil[2], Nigel Fullwood[3]
Departments of Chemistry[1] and Materials Science and Engineering,[2] Univ. Sheffield, and Biological Sciences, Lancaster Univ, UK

Statement of Purpose: Hydrogels are typically poor substrates for cell culture: without additional functionality human cells fail to adhere or proliferate. However, these water swollen materials can provide the closest synthetic analogues to the physical properties of the extracellular matrix. Although, cells do not adhere to hydrogels the addition of integrin binding peptides or hydrophobic domains can promote adhesion and spreading. An alternative to these strategies is to add groups that allow for enzymatic coupling of biomolecules that can promote adhesion. Potentially, the cells could provide the necessary enzymes and cell adhesion molecules. Using this concept we proposed that the provision of "lysine-like" functionality would provide a group that would react with extracellular enzymes, such as transglutaminases, which would allow the cells to modify the interface to allow adhesion. Also, other materials in general use as biomaterials often show poor cell adhesion properties that can be improved by adding alkyl amine functionality. Here we will show some simple routes (see scheme 1) for adding lysine-like alkyl primary amine functionality and we describe how a range of non-cell adhesive materials with various compositions can be modified to provide materials that were excellent substrates for cell culture.



Scheme 1 Preparation of hydrogels and oligomers containing alkyl primary amine functionality

Methods: Hydrogels were prepared by radical polymerization. Monomer mixtures were polymerized along with glycidyl methacrylate then the pendant epoxide groups were reacted with excess of diamine, as shown in scheme 1(a). Poly(butyl methacrylate oligomers were prepared by ozonizing poly(butyl methacrylate-co-butadiene) to give telechelic oligomers with carboxylic acid end groups. The end groups were then further modified with excess dialkyl amines as shown in scheme 1(b).

Results: Two sets of polymers with alkyl amine functionality were prepared: cross-linked methacrylate hydrogels and non-cross-linked oligomeric coatings. As shown in figure 1, cell adhesion to the alkyl aminated hydrogels was clearly a function of the structure of the alkyl group. The data show cell viabilities of human epithelial corneal cells (HCECs) in co-culture with bovine keratocytes (BK) cultured on hydrogels functionalized with various alkyl amines. These HCEC cells and other differentiated cells such as dermal fibroblasts, osteoblasts, chondrocytes and lung epithelial cells adhered and proliferated on hydrogels functionalized with alkyl amines but only if the alkyl chain was in excess of 3 carbons.

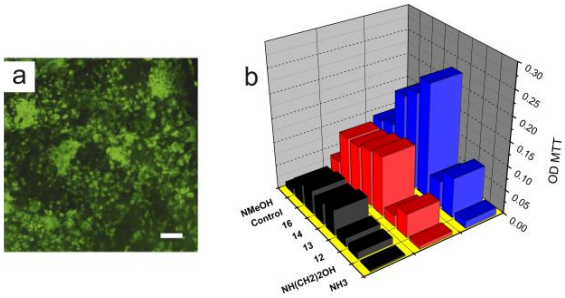


Figure 1 HCECs in coculture with BKs (a) labeled with K3 adhered and proliferating on a hydrogel functionalized with diamino butyl amine; (b) MTT cell viability after 3 (black), 6 (red) or 8 (blue)days culture. Hydrogels functionalized with: methyl amino ethan-2-ol (NMeOH); 1,6-hexandiamine (16); 1,4-butandiamine 14); 1,3-propanediamine (13); 1,2-ethandiamine (12); 1-aminoethanol-2-ol (NH(CH2)2OH or ammonia (NH3). The control is TCP coated with collagen.

The improvement of cell adhesion to preformed devices is also enormously important and an attractive strategy is to use conventional coating techniques with functional materials. Coatings with high degrees of alkyl amine functionality were produced by dip coating of poly(lactide-co-glycolide) meshes with aqueous dispersions of oligo(butyl methacrylate)s. As shown in figure 2 human dermal fibroblasts adhered to the coated material. Control experiments with non-coated meshes showed that virtually zero cells could adhere to the non-treated material.

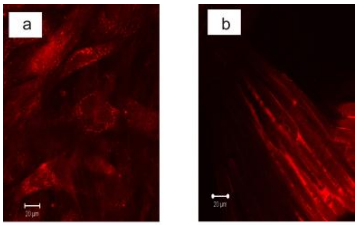


Figure 2 Human dermal fibroblasts labeled with red cell tracker adhered to (a) TCP or (b) a fibrous PLG mesh.

Conclusions: The addition of alkyl amine primary amine functionality to both solid and swollen materials (hydrogels) improves cell adhesion. There is a clear effect on the effectiveness of the chain length on cell adhesion with hydrogels but this effect is not observed when hydrophobic alkyl amine oligomers are used as coatings.