Engineering an Alginate Foam – A New Biocompatible Biomaterial

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Statement of purpose: There are numerous biomedical uses for biocompatible and biodegradable matrices made from biomaterials in the form of films, fibers, gels and foams. One suitable biomaterial with the desired physical and chemical properties is the biopolymer alginate. Alginate is linear polysaccharide found in marine brown algae as a structural component, and as exocellular material in some bacteria. Alginate is composed of the monomers β-D-mannuronic acid (M) and its 5-epimer α-L-guluronic acid (G) linked by ß(1→4) bonds. The relative content and intra-chain arrangement (sequence) of M and G, as well as the molecular weight and distribution, vary considerably from species to species. The chemical composition of alginate can affect the gel characteristics since gelling at physiological conditions involves cross-linking of alginate molecules by divalent cations mainly interacting with guluronate moieties in the polymer chains. A foam is created by stabilizing air in an alginate gel, and its properties related to biophysical characteristics can mainly be varied due to source and amount of alginate and gelling ions. This study presents different alginate foams and possible applications.

Methods: The alginate foam is created by mechanically agitating a dispersion of an aqueous solution of alginate, plasticizers, a foaming agent, a gelling agent and a slowly hydrolyzing acid. An insoluble gelling ion salt, *e.g.* $CaCO_3$, is used and Ca^{2+} ions are released by a reduction in pH induced by the hydrolysis of the pH modifier D-glucono-δ-lactone (GDL). The wet alginate foam is then cast as a layer or in a specific shape using a mold. The wet foam is kept at room temperature to allow complete dissolution of the gelling agent and then dried in an oven at 35-80°C. The amount of incorporated air and the foam's wet density are controlled by the mixing time. Varying the mixing time, foaming agent and gelling kinetics can control the pore size in the foam. Foam formulations were made using alginates differing in molecular weight and G-content, and with different sources of gelling agents. The particle size of $CaCO_3$ was also varied from about 1 µm to about 20 µm. In addition, the gelling ion was added in varying amounts sufficient to saturate the gelling sites of the alginate by 25-200%. The integrity of the foam was measured using an SMS Texture Analyzer with tensile grips after the dry foam was re-hydrated in a physiological solution. Cells were immobilized into the pores of the foam by dripping a cell suspension onto a dry foam patch. Some of the patches were post cell immobilization washed in an isotonic solution containing calcium ions before addition of cell growth medium. For quantification of cell growth was the foam patch dissolved by adding it into a solution containing a calcium-sequestering agent such as sodium citrate or EDTA. The cells were then retrieved and counted with use of a cell counting chamber (Bürker).

Results / Discussion: One of the main factors found to affect the gelling kinetics during foam formation was the particle size of the $CaCO_3$ used. The gelling speed increased as the particle size decreased. Several additional parameters were found to affect foam strength and stability: (1) alginate molecular weight, (2) alginate composition (G/M ratio), (3) calcium content, and (4) pore size. There was a relationship between foam strength and alginate molecular weight. However, at similar molecular weights, stronger foams were formed using a G-rich alginate than an M-rich alginate. Finally, foams saturated with calcium, or with excess amounts of calcium, were by far stronger than those formulated with sub-stoichiometric amounts of gelling ions. The pore size is typically in the range of 50-300 µm. Some major physical advantages of the foam are the high flexibility, the foam may be cut into specific shapes and sizes, it is not sticky and can easily be folded and refolded after hydration. Due to internal gel setting, the foams are also mechanically homogeneous and the structure can be sutured. Cells immobilized into alginate foams seems to grow well, and including an additional washing step in an calcium containing solution will accelerate the growth rate (see figure).

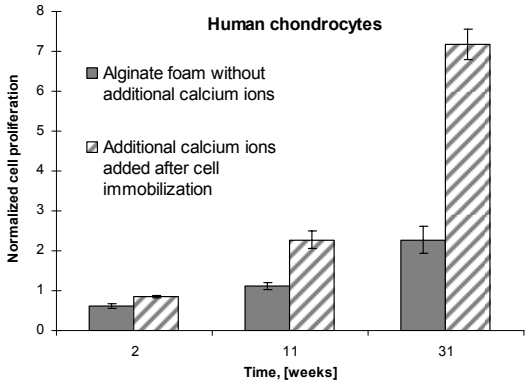

Figure: Proliferation of immobilized human chondrocytes.

Additional possible applications for an alginate foam can be as an anti-adhesion barrier, for wound management or as a system providing controlled release of drugs, antibiotics, growth factors etc. incorporated into the foam during the mixing step or added to the dry foam.

Conclusions: A biopolymer foam comprised of alginate was engineered whereby formulation flexibility in the choice of alginate and gelling ion characteristics could be used to construct foams having different physical properties. The alginate foam was also shown to be useful for *in vitro* cell culture by immobilizing cells within the foam pores. The flexibility in formulating this type of alginate foam can be used to advantage in developing numerous tissue engineering applications.