New injectable alginate formulation gels in contact with physiologic fluids

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Introduction: Alginates are natural polymers with advantageous properties as cell matrix materials within tissue engineering and regenerative medicine. In particular, the unique property of alginates to form gel structures in the presence of divalent cations under physiologic relevant conditions is useful allowing living cells to be entrapped within the gels. However, the rapid gel formation occurring in the presence of non-toxic divalent free cations like Ca^{2+} and Sr^{2+} may be limiting for formulations that needs to be injected or molded. We have, however, recently developed and internal self-gelling system allowing sufficient time for cells or other biomaterials to be mixed into the formulations before gel setting. In this work we have developed this technology further by making formulations with a very long delay before initiation of gel formation and shown that accelerated gel formation will occur upon addition of sodium ions.

Methods: Water insoluble gel formulations with delayed gelling time were prepared by mixing a sodium alginate solution with a dispersion of calcium alginate particles (Figure 1).

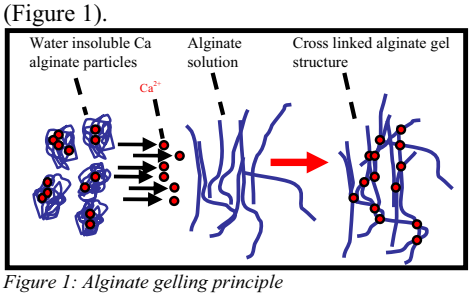

Figure 1: Alginate gelling principle

Mixing of the two components initiates gel formation as a result of exchange of calcium ions between the soluble and insoluble alginate fraction allowing the solutions to be injected or moulded before gel setting. The manufactured calcium alginate particles were manufactured at sizes typically around 45-75 µm and by using different manufacturing methods in order to vary particle structure. The alginate concentrations in the mixtures were typically around 2 %, and the properties of the gels were characterized by Bohlin rheology testing by means of measuring the storage modulus (G') as a function of time after mixing.

Results: By mixing the two components gelling was initiated and the buildup could easily be followed on the rheometer. In Figure 2 is shown different formulations previously developed. Through variation of gelling parameters the gelling rate and final gel elasticity may be varied giving a range of properties with respect to gel formation kinetics and final gel properties. In addition we have now found that the onset of gelling may be strongly delayed by changing the initial structure of the Ca-alginate particles through selected manufacturing methods. For such particles the delay in onset of gelling, however, only occurred when avoiding monovalent ions (Na^{+}) in the formulation. This is clearly shown in Figure 2 as an added physiologic level of sodium strongly promoted gel formation. Additionally, we also applied saline externally around the formulation on the rheometer after one hour then allowing diffusion of Na^{+} ions into the gel structure. The effect of non-gelling ions on gel formation is likely caused by their ability to facilitate exchange of Ca^{2+} ions between the soluble and insoluble alginate fractions. We have also performed similar experiments with cell culture media or serum as the source of gelling promoting ions with similar result obtained. Our data therefore consistently shows that self gelling mixtures may be formulated to be responsive to physiologic fluids. This technology may therefore offer new possibilities for injectable alginate based systems in a wide range of applications.

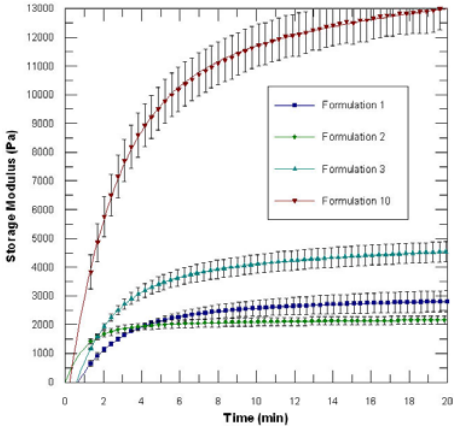

Figure 2: Storage modulus as a function of time for different alginate self-gel formulations.

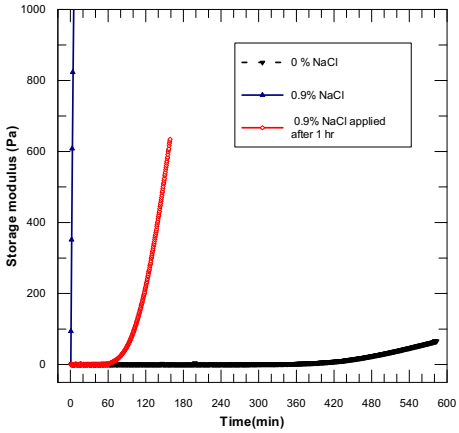

Figure 3. Storage modulus as a function of time for new self-gel formulations in the absence and presence of sodium either mixed in the formulation or applied around the formulation after one hour.

Conclusion: The self-gelling system developed may allow manufacturing of solely alginate based formulations with gel setting as a response to physiologic sodium ions.