New Alginate Self-gelling Technology for Tissue Engineering

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Statement of Purpose: Within the field of tissue engineering there is a demand for applicable biomaterials. Hydrogels made of alginate are promising in this respect because of unique properties and high degree of biocompatibility [1;2]. Alginates are non-branched biopolymers consisting of α-L-guluronic acid and its C-5 epimer ß-D-mannuronic acid that can be extracted and purified from seaweed or through bacterial fermentation [3]. A major feature of alginates rests in the gel forming ability under physiologic conditions in the presence of divalent cations. This principle is commonly used in the entrapment of cells or other biomaterials, and in particular for the generation of microbeads containing cells, drugs or other active biomaterials [1;2]. For microbead production the gelling system used, termed as diffusion gelling, means that the gelling ions diffuses into the alginate matrix from a surrounding reservoir. This process is very fast, as the surrounding gelling ions immediately initiates gelling only limited by diffusion. Although this may be an advantage in the process of generating structures like microbeads, the potential for controlling the gelling of other structures with desired shape and size is, however, limited. For the successful setting of such gel structures an alternative formulation is therefore needed. This may be accomplished by using internal gelling systems allowing the gelling ion source to be mixed with the alginate solution before the gels sets. In such systems the release of gelling ions from the source needs to be delayed sufficiently in order to allow proper mixing and handling. There are alternative methods for the production of alginate gels [4-7] including the use of a slowly reacting acid like D-glucono-δ-lactone to release calcium ions from calcium carbonate through pH reduction [4]. Currently available gelling systems may, however, have limitations because of low biocompatibility due to pH changes, involvement of other undesired constituents and sub optimal gelling properties. In the present work we describe the development of an alternative biocompatible self-gelling alginate system possessing the ability to control the setting time.

Methods: Self-gelling alginate formulations were made by mixing a dispersion containing insoluble calcium or strontium alginate particles with sodium alginate solutions. Insoluble calcium or strontium alginates of high purity were manufactured from PRONOVA™ alginates. Sodium alginate solutions were made from ultrapure PRONOVA™ alginates. Mixing was performed by either repeatedly forcing the solutions between two connected syringes, or alternately for small volumes by pipette mixing. After mixing, the gelling kinetics were studied and characterized by using a Physica MCR300 Rheometer using oscillation testing. The gel system was also studied by mixing cells into the matrix before gel setting. Cell viability and proliferation were observed with time using microscopy and fluorescence staining techniques. Furthermore, we also observed gel biodegradability with time through visual observations and compression testing.

Results / Discussion: Gelling of the system was initiated through rapid mixing of insoluble calcium or strontium alginate with sodium alginate solutions. This initiated a rearrangement of gelling ions between insoluble and soluble alginate molecules resulting in gel formation. The setting of the gel, as measured with a rheometer, was typically found to reach most of its stability within about one hour after mixing. In order to obtain optimal gel strength, however, mixing and shaping had to be finalized within about one minute after mixing was initiated. It was also found that the gelling process could be manipulated through variation of different parameters including: alginate concentrations and quality, concentration of gelling ions, insoluble alginate particle size, concentration of non-gelling ions, sequestrating agents and temperature. We also found that cells could easily be entrapped into the gel system without loss of viability and that the cells could be kept viable within the gel matrix for several months. Cell growth was shown to be dependent on the gel matrix formulation. It was also found that the gel system could be made more or less biodegradable by controlling, among other parameters, the concentration of gelling ions in the matrix.

We believe that this internal alginate "self-gelling"-system may find use within several biomedical applications, including implantation as a space filling material, coating of devices, drug or other active substance delivery applications, wound healing applications, cell entrapment as well as within tissue engineering applications among others.

Conclusions: The obtained alginate self-gelling system was found to have desirable properties as a shapeable biocompatible matrix with adjustable stability and biodegradability. The gel system may thus be adapted for use within different biomedical applications as a matrix for cells or others.

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