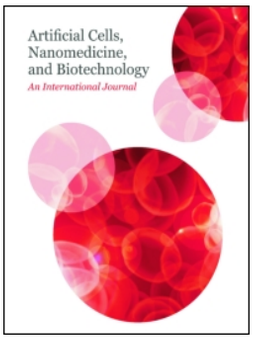

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Biocompatibility of Alginate

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LETTER TO THE EDITOR

Biocompatibility of Alginate

The application of semipermeable hydrogel microcapsules as immunoprotecting materials, in particular for cell transplantation and bioartificial organs, requires their careful design based on fundamental knowledge how component characteristics and preparation conditions affect the ultimate properties. Significant properties are, for example, mechanical stability, durability, permeability, and biocompatibility. Whereas mechanical and transport properties can be measured directly and quantified by physical parameters, biocompatibility is not at all a precisely describable characteristic for these microcapsules.

One of the most frequently used components of hydrogel microcapsules is the biopolymer sodium alginate. Although intensely studied for more than one decade, no general agreement exists concerning its biocompatibility in the scientific community yet. It is controversially under discussion if alginate with low or high percentage of manuronic acid units is advantageous, or if the molar mass has an impact. Furthermore, by several research groups the purity was found to have a remarkable influence. Recently, also the surface structure of microcapsules was reported as influencing factor. Therefore, all scientific studies are highly appreciated, which contribute to improve the understanding of alginates' biocompatibility.

The article "*Biocompatibility of Alginates for Grafting: Impact of Alginate Molecular Weight*," authored by S. Schneider et al. and published in Vol. 31(4), 383–394, of this journal presents the results of a study dealing with the biocompatibility problem. The concept of the

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study is interesting, however, the realization has some remarkable shortcomings.

- GPC calibration with neutral dextran standards, performed in this study, delivers much too high molar masses for the polyelectrolyte sodium alginate. Therefore, wrong molar masses are calculated from this calibration in the article. The MV Pronova alginates, such as used for this study, are known to possess weight average molar masses in the range of approximately 200,000–300,000 g/mol and not in the range of over one million as presented in the article.
- The authors use sterile solutions but nothing is indicated about the purity. The authors mention the importance of impurities such as endotoxins or mitogens with even some references but there is no information concerning the purity of their samples. It is well known that sterile filtration can only reduce the level of these impurities to a certain extent, which is, in general, not sufficient at all. Moreover, the degradation procedure applied in the paper is known to increase the endotoxin level in alginate samples. It has, therefore, to be demonstrated by analytical data that the biocompatibility really results from the lower molar mass and not from a higher endotoxin level after degradation.
- The unit of the dynamic viscosity is ''mPa s'' and not ''mPa/s'' as used in the article exclusively several times.
- Table 1 does not present the ''molecular weight distribution'' but simply average values of the molar mass. In addition, the authors use several times only ''weight average'' instead of the precise expression ''weight average molar mass.''
- The information from Fig. 3 is in contradiction to the information of Fig. 4 as well as the explanation and values given on page 391 explaining Fig. 3.
- The conclusion that alginate below 100,000 g/mol has to be removed in order to increase the biocompatibility cannot be drawn in this absolute way by the experimental data. Maybe there is a negative influence of oligomeric alginate but this has to be specified by precise macromolecular characteristics of purified samples and has, in addition, to consider the chemical composition (G/M ratio) of the alginate. Moreover, oligosaccharides have much lower molar masses than 100,000 g/mol.

As outlined in the introducing paragraph, the biocompatibility of alginate and its influencing factors are of such high importance that we

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should avoid nonprecise conclusions which may prevent scientific progress.

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LETTER TO THE EDITOR

To The Editor

Over the past two decades many different hydrogels and synthetic polymers have been proposed for immunoisolation and encapsulation of cells. Among these, alginate is one of the most promising natural polymers as demonstrated by animal studies and small-scale clinical trials (Orive et al., 2003). But, a major problem for encapsulated cell/tissue transplantation is adjusting numerous matrix- and host-related parameters to achieve immunoisolation without impeding the release of factors generated in the microcapsules core. The biocompatibility of the used alginate is one of the crucial issues which have to be addressed when designing an encapsulated transplant for clinical application. But, as mentioned no general agreement exists concerning its biocompatibility and the question if the molar mass has an impact. On the basis of these data, it was mandatory to address this issue in a study to increase scientific progress in this emerging field.

In the method section (Page 388) it was clearly stated why the GPC-analysis was performed with dextran—instead of alginate-standards. Therefore, it is obvious that relative—and not absolute—values for the weight average molar masses were presented. In this context, it is clear that the absolute weight average molar mass of the used alginate preparation differs from the exact presented relative values. However, for the performed experiments it was not necessary to measure absolute values as suggested by Wandrey, but to show that significant differences exist concerning the average weight molar masses of the used alginate samples and this was the case. Furthermore, our analytical data do not support the hypothesis that the endotoxin content of the MV-alginate increases by the degradation procedure and, in turn, is responsible for the reduced biocompatibility. The endotoxin level of the used alginate is known to be below 100 EU/g and remains there after degradation. In this context the data clearly showed that the biocompatibility of capsules increases with the weight average molar mass of the used alginate. Concerning Fig. 3 we have to apologise, because a failure occurs with

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the lettering below the columns (corrected version: MV-alginate [black column] and LV-alginate [grey column]). In addition, we would agree that the conclusion concerning the cut-off ($<10^{-5}$) should be drawn more careful, but in general low molecular weight fractions should be eliminated to increase biocompatibility.

On the basis of the presented data (Schneider et al., 2003a), we are completely convinced that the biocompatibility of alginates significantly increases with their weight average molar masses. Therefore, we switched from commercial available MV- or LV-alginates (Pronova, Drammen, Norway) (Schneider et al., 2001, 2003a, b) to highly purified ultra-high viscosity alginates (UHV; Lehrstuhl für Biotechnologie, University of Würzburg) for ongoing investigations (viscosity of a 0.1% w/v solution in distilled water for LV-alginate: 1–2.5 mPa s vs. MV-alginate: 5–7 mPa s vs. UHV alginate: 20–30 mPa s) (Schneider et al., 2003c; Zimmermann et al., 2003). The extraction and purification protocol of UHV-alginates, for which medical approval is granted, is described elsewere (Leinfelder et al., 2003). We have recently shown that use of UHV-alginates when cross-linked internally and externally together with Ba^2 resulted in micro-capsules of extremely high stability, particularly when proteins (e.g., human serum albumin) are incorporated simultaneously (Schneider et al., 2003c). First studies have shown that rat islets encapsulated in these novel microcapsules exhibited a well-preserved insulin secretion in tissue culture over three weeks. Furthermore, these microcapsules protected rat islets against xenogenic complement-dependent components (Zimmermann et al., 2003). In the meantime a novel class of UHV-alginates with a very high biocompatibility is available that results in long-term survival (>7 month) of adult rat islets when encapsulated in these novel microcapsules and transplanted into immunocompetent diabetic mice without the need of immunosuppression (unpublished data).

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