

## 5 Summary

The present contribution describes the preparation of protein loaded, biodegradable microparticles from poly(lactide-co-glycolide) [PLGA] using a new, reproducible  $w_1/o/w_2$  double emulsion solvent evaporation technique based on a micromixer under aseptic conditions. In the micromixer the size of the resulting particles could be controlled and thus particles of optimum size for the phagocytosis by dendritic cells (DCs) were obtained. The  $w_1/o$  primary emulsification of the aqueous solution of the model protein FITC-BSA in the organic solution of the PLGA matrix polymer has been optimized under consideration of the ultrastructure of the microparticles, the protein distribution, as well as the release behaviour. The microparticles have been characterized comprehensively.

Without checking the real residual content of solvents in the microparticles occasionally the literature suggests preparation procedures on using alternative solvents, for which the pharmacopoeia allows higher residual concentrations. In the present investigations it could be proved, that the residual dichloromethane in the microparticles, prepared with the presented preparation technique, was one thousand times smaller than required in the pharmacopoeia. Thus the search for alternative solvents is not necessary.

Within the scope of this presentation the usage of FITC-BSA was evaluated concerning its suitability as a model protein in microencapsulation. By means of fluorescence labelling qualitative examinations of the protein distribution in the microparticle and the localisation of the particle in the cell experiment were made, which allowed a number of conclusions with respect to the ultrastructure of the particles and their phagocytosis, respectively. The fluorimetric quantification of FITC-BSA in samples of release studies failed, thus other methods for the protein quantification had to be developed and accordingly established. The reason for the increasing fluorescence under release conditions had been extensively examined and recognized to be a dequenching effect due to a minor protein hydrolysis. It is a basic phenomenon of multi-FITC-labelled proteins and can be applied to other fields (e.g., immunofluorescence), too.

In cell experiments it could be shown that there is an efficient uptake of the microparticles, prepared on using the micromixer, and the intracellular localisation could be proved unambiguously by means of different techniques. Simultaneously with other scientists it could be shown for the first time, that in the absence of endotoxins or other strong antigens PLGA microparticles do not initiate the maturation of DCs or changes in the expression of

their surface markers. This confirms the absence of endotoxins, determined by the LAL test, and demonstrates the success of the aseptic preparation procedure.

A method of cationic surface modification of PLGA particles using cetyltrimethylammonium bromide (CTAB), as described in the literature, could not be reproduced. The desorption of the CTAB from the particle surface had been found to be the reason for the instability. In an alternative procedure DEAE dextran has been used for surface modification of PLGA microparticles for the first time. Thus stable cationic microparticles could be produced. Contrary to the surface modification with Chitosan as suggested in the literature, the DEAE dextran coated particles had a positive surface charge even at pH 7 due to the high  $pK_a$  value of the coating. During the final purification steps the surface charge of the particles and the concentration of cationic stabilizer were checked. The residue of excess cationic stabilizer in the microparticle suspension had been reduced below the toxicity level on retaining the stability of the formulation.

The uptake of DEAE dextran modified particles by phagocytising cells was more efficient than that of non-modified particles. The phagocytosis of the surface modified particles did not lead to a change of the DC phenotype, too. Thus, in agreement with recent data of other scientists, previous contradictory papers could be disproved, which postulated an increased expression of different surface makers by phagocytosis of anionic and cationic particles, respectively, most probably due to a contamination with lipopolysaccharides. The phagocytosis of surface modified or non-modified particles did not lead to cytotoxicity under the used concentration of the particles.

Thus PLGA microparticles are safe carrier systems to deliver antigens to dendritic cells. For an improved immunological cancer therapy antigen pulsing of autologous dendritic cells with microencapsulated antigen should be carried out. The novel surface modification of microparticles with DEAE dextran allows the adsorption of anionic ligands of Toll-like receptors. This might control the pathway of the further immune response and might lead via reduced back-differentiation of dendritic cells *in vivo* to further improvements of the efficiency of DC based cell therapies.