Nano- and Microspheres for Pharmaceutical and Medical Applications

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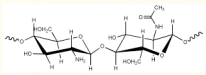
Introduction

Biopolymers such as polysaccharides and proteins play a central role in life sciences. Function and application are generally based on electrostatic interactions with oppositely charged molecules or surfaces. However, the latter and the related structure - property relationships are still far from being fully understood. Characterization and control of these are essential to establish efficient and successful therapeutic benefits. Furthermore, comprehension of the particle - cell interactions are crucial in order to understand, enhance and develop new therapies.

Chitosan-based Hydrophilic Nanoparticles

Chitosan is a natural positively charged polysaccharide. Its biodegradability and biocompatibility makes it an interesting polymer for biomedical applications. Chitosan is derived from chitin by a process of deacetylation.



Chemical structure of chitosan

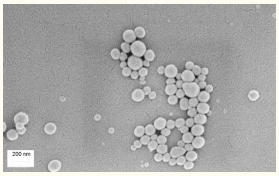
Nanoparticles are based on chitosan and other polysaccharides, natural biopolymers known for their biocompatibility. The process of nanoparticle formation is entirely water-based. During formulation process surface properties are designed to address specific cellular targets. Drug or other molecule incorporation is possible during the formulation process.
This project is undertaken in collaboration with Medipol SA (Ecublens, Switzerland) which has developed these nanoparticles[1].

Characterization

Chitosans from two sources - animal and fungal - are purified and characterized in terms of chemical nature, molar mass, intrinsic viscosity and degree of deacetylation. The influence of these raw material properties on the nanoparticles formation and characteristics will be studied.

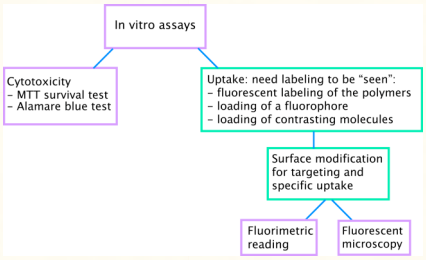
Size distribution and surface properties are the main characteristics of the nanoparticles, in particular for further applications in biological environments. However, due to the polymeric and hydrogel nature, limitations occur in the methods of characterization.

Morphology and size distribution can be assessed by **electron microscopy**. SEM and TEM imaging are performed on dried nanoparticle suspensions. Due to their hydrogel type, it is important to image the nanoparticles in their hydrated form. This is possible with e.g. cryo-TEM. The latter will be a powerful tool in the nearer future to asses the intact and real morphology in suspension of the nanoparticles.



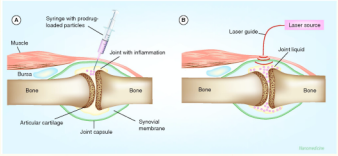
Electron microscopy (SEM) picture of chitosan loaded nanoparticles. Dried from water based dispersion, platinum sputter coated.

***In vitro* experiments:**
In vitro experiments are performed in collaboration with the group of Dr. L. Juillerat-Jeanneret, Institute of Pathology, CHUV, Lausanne, Switzerland.



Chitosan Nanoparticles in Biomedical Applications

Chitosan based nanoparticles have been recently shown to enhance the delivery of photosensitizer into inflammatory cells for rheumatoid arthritis treatment by photodynamic therapy (PDT).



Photosensitizer is loaded in the nanoparticles. The latter are then injected in the joint. Phototoxicity is induced by action of light.

Outlook & Perspectives

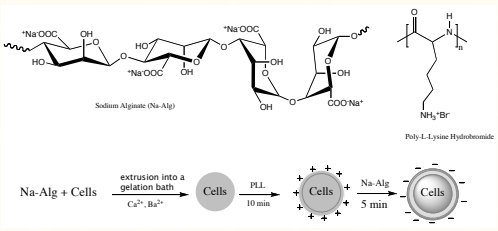
- Detailed characterization of the nanoparticles
- Influence of the raw materials and nanoparticles process
- Stability studies in various biological environments
- Establishment of nanoparticle properties - particles/cell interaction relationships
- Whole body imaging of labeled nanoparticles *in vivo*

Alginate-based Microcapsules

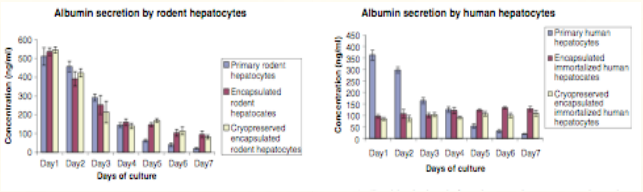
Sodium alginate is one of the most widely used polymers for cell entrapment. Hydrophilic microcapsules formed from alginate find many applications in the biomedical field. The microcapsule walls protect the cells from the immunological reactions and maintain their viability by allowing diffusion of oxygen, nutrients, and metabolic products.

Treatment of Fulminant Liver Failure

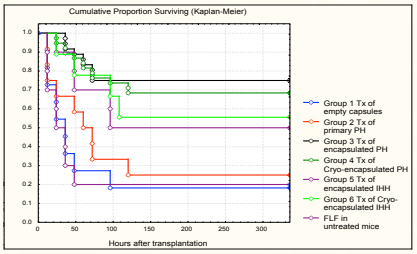
This work has been done in the group of Prof. Léo Bühler, Department of Surgery at HUG. Rat and immortalized human hepatocytes (IHH) were encapsulated in 400 µm alginate-PLL-alginate membranes and cryopreserved. In vitro, albumin production was measured by enzyme- linked immunosorbent assay (ELISA).



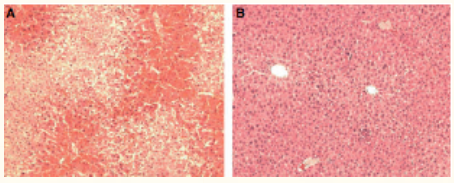
At day 0 of culture, IHH scattered homogeneously in capsules (A, 40x). At day 3 after culture, IHH attached together to form "cellular masses" (B, 63x). After 1 (C, 40x) and 2 (D, 63x) weeks of culture, hepatocyte masses were still growing, and trypan blue exclusion staining of cell masses showed that only few cells were staining in blue color on the surface of cell masses, but no core necrosis was detected(E, 100x). Capsules containing IHH were collected from the peritoneal cavity of experimental mice 10 days after induction of FLF and transplantation. These encapsulated IHH were put back in culture and showed continuous cell proliferation (F, 40x).



Cryopreserved or fresh encapsulated rodent hepatocytes showed a progressively decreasing albumin secretion over 1 week in culture. In contrast, cryopreserved or fresh encapsulated IHH showed minimal, but stable albumin secretion.



Delivery of cryopreserved encapsulated cells (Group 4) similarly improved survival (increased survival rate of mice with FLF from 20% to 68%), demonstrating that cryopreservation did not significantly alter hepatocyte functions.



Histopathology of native liver tissue demonstrated massive hemorrhage and extensive hepatocytes necrosis at 15 h after acetaminophen administration (A, hematoxylin-eosin,100x). In contrast, surviving mice showed complete recovery of native liver tissue 2 weeks post-transplantation (B, hematoxylin-eosin, 100x).

Outlook & Perspectives

- Development of new materials for cell encapsulation using biopolymers, and/or modified alginate
- Improvement of biocompatibility and mechanical stability
- Establishment of parameter-properties relationship

References

[1]WO2007/031812 (PCT patent);

Mai et al, Xenotransplantation 2005: 12: 457-464

Acknowledgements

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