Study of surface adsorption on sodium alginate/poly-L-lysine beads by M-FTIR

A. Rossi[1], B.J. de Haan[2], F. Sonvico[1], P. de Vos[2], P. Colombo[1]

[1] Department of Pharmacy, University of Parma, V.le G.P. Usberti 27/A 43100 Parma, I
[2] UMCG, Phatology Laboratory Medicine, Hanzepleinn 1, 9713 GZ Groningen, NH

INTRODUCTION: A requirement for sustaining long-term viability and function of transplanted cells is to use microcapsules that are biocompatible and induce minimal inflammation. The immediate adsorption of proteins from biological fluids onto an implanted biomaterial is believed to govern all subsequent cellular responses to the implant, including inflammation. This work investigated the adsorption of protein from human serum and human peritoneal fluid to the surface of alginate/poly-L-Lysine microcapsules (APA) by means of Micro Fourier Transformed Infrared Spectroscopy (MFTIR).

MATERIALS: Sodium alginates with 'intermediate' guluronate (Keltone) or 'high' guluronate content (Manugel®) and Poly-L-lysine (PLL) HCl were used. Human serum and human peritoneal fluid were both obtained from healthy volunteers.

METHODS: Droplets of sodium alginate in KRH medium were produced using an airjet system. The droplets were gelled in 100 mM $CaCl_2$ to form beads that were subsequently immersed in PLL for 10 min then in sodium alginate for 5 min to produce 'APA' microcapsules. Samples of microcapsules were incubated in dilute human serum (HS) or human peritoneal fluid (HPF) for 1h a 37°C, then rinsed 5 min with KRH.
The MFTIR spectra were collected at room temperature in the 4000-650 cm^{-1} wavenumber range in transmittance mode. Sodium alginate beads and sodium alginate beads with a PLL layer, with and without incubation in HPF or HS, were placed on the top of KBr disk. The samples were analyzed by setting the microscope aperture on the marginal site of the bead. As comparison, MFTIR spectra of sodium alginate, PLL and of human proteins were collected.

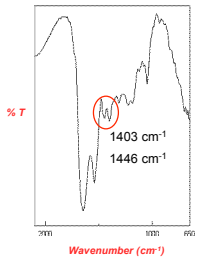
RESULTS

As both human serum and human peritoneal fluid are complex systems, it is impossible to attribute the MFTIR spectrum to an individual component. Some characteristic peaks were identified: two peaks at 1446 and 1403 cm^{-1} in the case of HS and four peaks at 1664, 1639, 1232 and 1178 cm^{-1} for HPF.

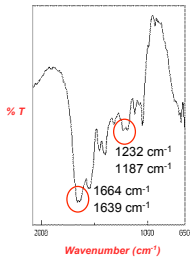
MFTIR spectra of Keltone and Manugel alginate beads show a broad absorption band at around 3370 cm^{-1} for the –OH groups, two peaks at 1605 and 1417 cm^{-1}, both related to the $-COO^-$ groups and an absorption band between 1200 and 1000 cm^{-1}, corresponding to the vibration of C–O bond.

The incubation of the Keltone beads with HS resulted in the appearance of two peaks in the MFTIR spectrum at 1446 and 1403 cm^{-1}, characteristic of the proteins. On the other hand, after incubation of the Keltone beads with HPF no differences in MFTIR spectrum were observed.

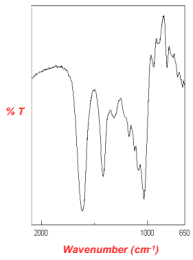
Human serum



Peritonal fluid

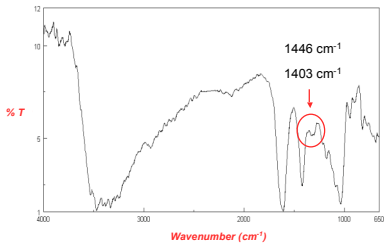


Alginate beads



Also in the case of Manugel beads, incubated with HS, significative differences in the spectrum were noticed. The incubation resulted in the appearance of two peaks at 1618 and 1211 cm^{-1}. The beads showed also a different pattern after the incubation with human PF, resulted in the broadening of the shoulder in the MFTIR spectrum around 1200 cm^{-1}.

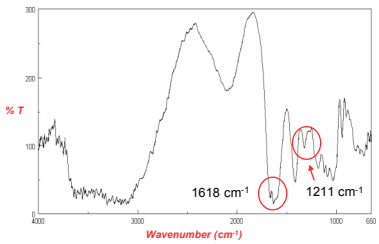
Incubation of Keltone beads with HS



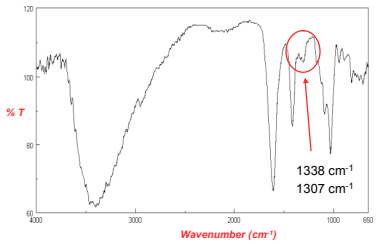
The MFTIR spectrum of APA capsules of Keltone and Manugel showed an absorption band at around 1625 cm^{-1}, corresponding to the C=O stretching, which is reinforced and broadened by both the contribution of the amidic C=O in PLL and the carbossylic C=O in sodium alginate. The absorption band, corresponding to the NH_3^+ group of PLL, shifted from 2020 to 2183 cm^{-1}, likely due to the interaction of NH_3^+ of PLL with COO^- of sodium alginate.

Incubation of the Keltone APA with HS resulted in a shifting of the two peaks, related to the HS proteins, from 1446 and 1403 cm^{-1} to 1338 and 1307 cm^{-1}. Incubation of the Keltone APA with HPF resulted in the appearance of four peaks at 1664, 1599, 1369 and 1332 cm^{-1}. In the Manugel APA capsules the incubation with HS resulted in the appearance of four peaks in the MFTIR spectrum at 1633, 1610, 1338 and 1313 cm^{-1}; while the incubation with human PF resulted in the appearance of four peaks in the MFTIR spectrum at 1664, 1619, 1216 and 1174 cm^{-1}.

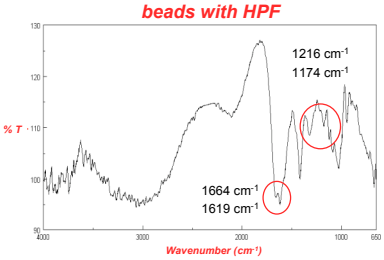
Incubation of Manugel beads with HS



Incubation of Keltone APA beads with HS



Incubation of Manugel APA beads with HPF



CONCLUSION: MFTIR technique is a powerful tool to study the adsorption of human serum and human peritoneal fluid components to alginate based capsules. A shift in the characteristic peaks of both tested human fluids was observed. Only the Keltone bead was free of HPF and showed minor protein adsorption. The spectra of Manugel beads were altered due the presence of HS and HPF. The adsorption was depended on the alginate type and the presence of PLL at the surface. Both APA capsule types had measurable differences in MFTIR spectra due adsorption of HS or HPF at the surface.

ACKNOWLEDGEMENTS: The authors acknowledge COST865 project for the support.