## Free-standing multilayer films made of chitosan and alginate for biomedical applications

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Objective. The method for preparing multilayer films by the consecutive deposition of oppositely charged polyelectrolytes has gained tremendous recognition due the user friendly preparation, capability of incorporating high loads of different types of biomolecules in the films, fine control over the materials' structure, and robustness of the products under ambient and physiological conditions. However the preparation of such films needs the assembly on a substrate and, sometimes, cannot be detached from it, which has limited the application of such films in areas as tissue engineering and regenerative medicine. Herein, we report the production of chitosan/alginate (CTS/ALG) free-standing films that can be detached from an underlying inert substrate without any postprocessing step allowing the determination of physical properties of fundamental significance such as ion permeation and mechanical properties.

Methods. In this work, the buildup of free-standing multilayer films made of CTS and ALG was investigated. Several conditions were tested to follow the film growth in order to get thick films. The CTS/ALG free-standing films were characterized by Fourier transform infrared spectroscopy (FTIR) and by scanning electron microscopy (SEM). Permeability tests were performed using FITC-dextran, with several molecular weights, as a drug model molecule. Cell adhesion was assessed using C2C12 myoblast cells over a period of 48h.

Results and Discussion. The produced membranes can be detached from an underlying inert substrate without any postprocessing step. Permeability experiments on these membranes revealed that the permeation of FITC-dextran depended greatly on its molecular weight. The results showed that these films are a good substrate for cell adhesion as spread cells were observed all over the surface, by actin and heochst staining.

Conclusions. The production of free-standing films permits the direct experimental determination of many physical properties of fundamental significance such as ion permeation and mechanical properties that can be tuned for real-world applications. These free-standing films are easy detachable, easy to handle, stable in the presence of physiological solutions and biocompatible, demonstrating potential for applications in tissue engineering and regenerative medicine.