



CHITOSAN FILM PREPARATION
Instructions for laboratory experiments

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1 INTRODUCTION

Chitosan film is relatively easy to prepare, but difficult to handle due to curving during drying phase, if no assisting means and weights are used to keep it straight. In liquid it becomes soft and swells, which makes feasible research hard. This report contains information for the preparation of a chitosan film sample and the use of CellCrown cell culture insert as assisting device for this experiment.

Microcrystalline chitosan powder was used in these experiments. Other types of chitosan, for example medical grade chitosan powder, may behave differently when using the same recipe and this should be taken into account when planning the testing. According to earlier experience the film preparation using medical grade chitosan powder might be more challenging, ending up to brittle and fragile film.

Chitosan is soluble in mild acids as acetic acid, lactic acid, citric acid, hydrogen chloride solution and formic acid. The properties of the films may vary when different acids are used. Acetic acid was used in these experiments.

2 EQUIPMENT AND MATERIALS

Microcrystalline chitosan powder

Distilled water

Acetic acid ($\text{CH}_3\text{-COOH}$)

5 Mol Natrium Hydroxide (NaOH)

Pipette

250 ml decanter glass

Laboratory mixer

Petri glasses with diameters of 30, 50 and 90 mm and 6- and 24-well plates

Surgical knife

Pinch

CellCrown6, CellCrown12 and CellCrown24 inserts

Fume chamber

3 RECIPE

2 g Microcrystal chitosan powder

2 ml Acetic acid (CH₃-COOH)

100 ml Distilled water

Put chitosan powder and distilled water in a 250 ml decanter glass and mix, until the solution is homogeneous. Add acetic acid and continue mixing until the liquid is homogeneous and clear, which takes around 0,5 - 1 hour. Then measure the wanted amount of solution on petri glasses or in the wells of a well plate. The viscosity of the chitosan solution is quite high, as syrup, and therefore a measuring glass is practical for the measurement of the wanted volume. The following table 1 contains the information of the tested dishes, used volumes of chitosan solution, and the thickness of the final chitosan film, accordingly.

Table 1. Volumes of chitosan solution for film preparation

Ø (mm)	Inner height (mm)	Volume (cm ³)	Volume of used chitosan solution (ml)	Thickness of final film (µm)
30	10	7,065	5	260-290
50	18	35,325	30	780-800
90	10	64,585	50	245-280
15*	15	2,649	1	70-80
15*	15	2,649	2	130-150
35**	15	14,424	10	170-220

* well of 24-well plate

** well of 6-well plate

Place the glasses with chitosan solution in a fume chamber for at least 24 hours. After this drying phase put 5 Mol natrium hydroxide (NaOH) liquid onto the chitosan film for 5 – 10 minutes. It helps in the removal of the chitosan film from the petri glass. During the drying phase the chitosan film forms also onto the edge of the petri glass and the walls of the well as well as on the bottom. Use a surgical knife to cut between the wall and the film and try to maintain the cylinder shape of the film (Figure 1). This helps in the removal of the film as an entity from the glass and later in the attachment with CellCrown. Then neutralize the film by bathing it in distilled water.

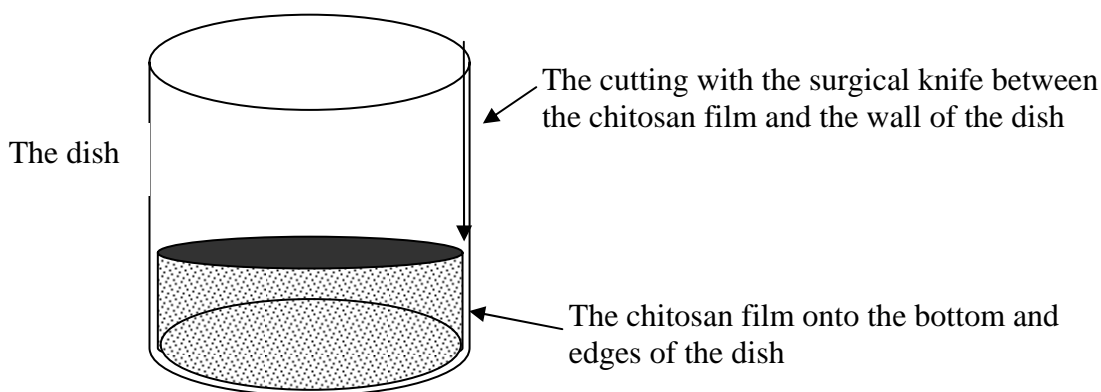


Figure 1. Removal of the chitosan film from the dish

Now the chitosan film is ready for installation in the wanted size of CellCrown. The thicker films (above 250 μm) are more difficult to install as round samples and they need extra weight on top even when CellCrown is used as an assisting device. Another option is to cut a ribbon of chitosan film, which in width matches the diameter of the body of the CellCrown (Figure 2). This kind of sample is easier to attach and handle with CellCrown, when the sample is rigid or thick.

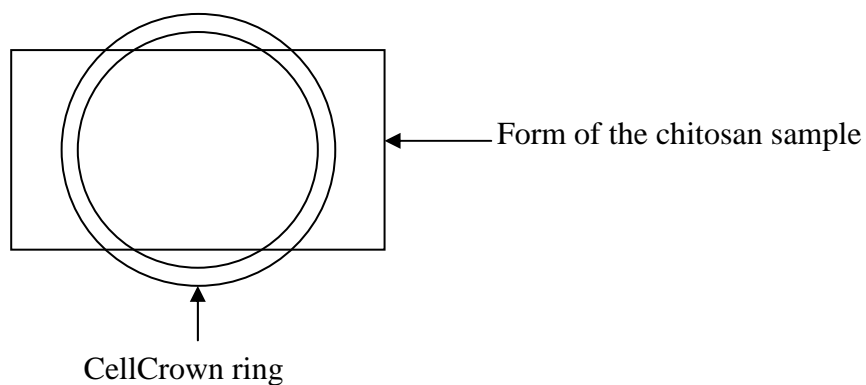


Figure 2. The form of the rigid and thick samples

It was noticed during the experiment that the chitosan film was the easiest to handle when the thickness was between 120 – 200 μm . Below 100 μm it requires very gentle and careful handling. Also when chitosan solution is dried in 6- and 24-well plates, the final film is easy to attach with CellCrown6 and CellCrown12. With those samples it was possible to place the ring

part of CellCrown into the bottom of the well plate, put the film on top of it and push the body of CellCrown on top of the film.

It is possible to use the chitosan film immediately for the wanted tests or to dry it in a CellCrown insert and use it later. Upon drying of the chitosan film some weight is needed on the top of the CellCrown or it needs to be pushed down in the bottom of the well plate. When wetted again, the chitosan film becomes soft and swells. Therefore, the CellCrown needs potentially to be opened, the film straightened again, and the ring put back in place.

Sterilization of chitosan films is still a challenging question. Autoclaving destroys them even when autoclaved with a CellCrown insert. Also other sterilization methods are investigated by Scaffdex, but at the moment the only sterilization option, which is known to be safe for the chitosan film sample, is rinsing it with ethanol.