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1. Protocols followed

1.1 Cellular adhesion protocol (L132)
Fill the cells at least 48 hours before within growth medium (T25 or T75)

Day of manipulation:

- Preparation of growth cell mediums (COSTAR 24 well plate rings) for 30, 60 and 120 minutes. Put the samples of membranes in the bottom of well plate with or without a retaining ring (black rings of cytocompatible rubber).
- Prepare the growth cell medium (MEM of Eagles+ Fungizone and Gentamicine) to a concentration 5% of SFV.
- Removing cells from the growth medium
  o Rinse with a few of EDTA
  o Put 0.5 ml of trypsine (T25) and 1 ml (T75)
  o Put on a incubator at 37ºC during 10 min
  o Introduce 7 ml (T25) to 10 ml (T75) of growth
  o Rinse the pot (plate) and clear out the solution within a pot for centrifuge
  o Centrifuge 12 min at 650 rpm
  o Clear out the remainder (there are still cells pasted on the bottom of tube) and take it back with 10 ml of medium (put the cells in suspension)
- Counting cells with the cell of MALASZE
  o Count 2 lines from 10 lines and multiply by 5 and by 1000 in order to have the concentration of cells per ml.
  o Make the accurate dilution to have a concentration of 100 000 cell per ml
  o Count on the cell of MALASZE (6 times from the total of grid pattern ), do an average
- Drop off 0.6 ML of medium in every tube
- Introduce 0.4 ml of cell solution in order to have 40 000 cells per tube
- Leave it in incubator throughout 30, 60 and 120 minutes.

After 30, 60 and 120 minutes

- Clear out the tubes of growth cell medium.
- Rinse 2 times the tubes using PBS (preparation of PBS explained below)
- Add 300 µl of pNPP in each tube (preparation of pNPP explained below)
- Cover with aluminum foil
- Leave it in incubator during 3 hours at 37ºC
- Stop the reaction with 150 µl of soda (preparation of soda explained below)
- Introduce 200µl of each tube in a box of 98 tubes completely transparent
- Read number with reader ELISA 405nm (specifications in machine)

1.2 Preparation of solutions

**PBS**

Dissolve a pastille of PBS in 200ml of distilled water

**Preparation of acetate tampon**

22 ml acetic acid 0.2 M (11.55 ml in 1L)
86 ml d’acetate of sodium 0.2M (27.2g in 1L)
108µl of triton

Adjust until pH 5.5 with acid or acetat of sodium

**Preparation of pNPP**

Make a solution with 3mg/ml of pNPP. Before, the volume needed must be calculated in order to do not exceed.

Take 3mg of pNpp (from the freezer) and add it in 1ml of solution of tampon acetate pH 5.5

Put the aluminum around.

**Preparation of soda**

Prepare a solution of soda 1N ( 1 mol/L and 40g/L)

Take 4g of soda and dissolve in 100 ml of distilled water.

Soda does not persist after 1 month. Thus, it is recommended to make only the solution required.
**Surface modification of textile PET (not included)**

**Preparation of samples**

Cut a sample of textile with dimension about 5x10 cm²

Clean with soxhlet par (isopropanol and then distilled water)

Drying in a sterilizer during 1h at 90ºC

**Cross-linking reaction and coating of textile**

Preparation of a solution with cyclodextrine and citric acid: CTR/NaH₂PO/HPβCD with a mass ratio (8/1/10) using distilled water as solvent.

Impregnate 8 times the textile PET through solution.

Dry at 90ºC throughout 7min.

Thermal-fixation at 140ºC throughout 1h

Clean with water in order to eliminate residues.

Neutralization by using sodium carbonate 4g/l throughout 15 min.

2. Methods failed

2.1 Cross-linking of PEO with acid citric (CTR)

Both polymers are mixed and dissolved in distilled water. Different ratios of mass have been tested but in all cases the production of the membrane by electrospinning is found inappropriate. For different concentrations of mass in solvent between 2%- 3% of PEO and 2% -3% of CTR, the membranes carried out by electrospinning shows thin fibers and humid surface.

With a percentage of 4% PEO and 1% CTR, the membrane resulted was slightly improved but it was found weakly even after thermal cross-linking. As we manage producing an electrospun membrane with only PEO as a solute (3-4%), we conclude that the problems might come from the concentration of CTR or the reaction between both polymers.

The currently crosslinking of PEO and CTR has been found to be inaccurate for the present project, so that another way of crosslinking was reconsidered. From the article found, a change in methodology was decided and our planning was readapted. The solution has been putted into ultraviolet treatment. Among different solvents such as acetone, alcoholyl, ethyle and acetonil, acetonil was found the only dissolvent which is able to
dissolve PEO. Acetonil was used as solvent with water in a proportion of 50/50 and meshed to PEO.

After that, the photoindicator Darum was added in catalyze the cross-linking between PEO-CTR. As some photoindicators, Darum is sensible to the light. Thus, by the moment that Darcur is added to the UV treatment, any single contact of solution with light must be avoided. Electrospinning process of PEO was performed in dark conditions. However, results has been failed either. Another cross-linking method was considered. However, we conclude that further experiments have to be tested whether the cross-linking between darum and PEO is possible.

2.2 Surface modification: functionalization

When a material is functionalized, it means that chemical functional groups are added to its surface. The main purpose of this procedure is to add cyclodextrine in the surface of mesh. Due to its interesting properties, it has been proven that cyclodextrine are an excellent drug carrier system (ref: Cyclodextrin Drug Carrier Systems). As it has been explained in the introduction of the present project, after achieving our main objective this membrane developed has to be used as drug delivery system beyond its anti-fouling properties.

Then, our solutions have to be adhered at the surface of this functionalized mesh. In order add the solution onto the textile, we have attempted to change the electric surface of mesh, which is neutral in standard conditions, into negative polarity. Thus, an interaction between chitosan (with positive polarity in its surface) could be induced so as this bond might fix our solution into the mesh.

Our method follows the procedure done in an article issued by some of the researchers here in our laboratory (Finishing of Polyester Fabrics with Cyclodextrins and polycarboxylic Acids as Crosslinking Agents). They succeeded in grafting cyclodextrines (CDs) particles onto PET fibers by the intermediate of polycarboxylic acids that played the role of crosslinking agents. They concluded that the mode of grafting occurred through the formation of a crosslinked copolymer between PCA and CDs. This copolymer was physically adhered or was entangled into the fibrous network so that grafting was resistant to washings and was permanent. By testing three different cyclodextrins, HP-β-CD was found to be the one with lowest reaction time.

The figure below represents a scheme of the reaction between both polymers.
Sodium dihydrogen hypophosphite (NaH2PO2) was used as catalyst. In fact the covalent reaction between both polymers is an esterification. Carboxylic acid is engaged with alcohol group of cyclodextrine forming an ester.
From the three different polycarboxylic agents tested, we have chosen citric acid and HP-β-CD as cyclodextrin agent. Two types of polyester have carried out: Polyethylene terephthalate (PET) and Polylactic acid (PLA). Mass ratio among HP-β-CD, CTR and NaH2PO2 is 10/8/1 respectively. Polyesters were impregnated by the aqueous solution. After impregnation, meshes were dried at 86ºC through 15 minutes in order to remove the excess of water. Then, mesh was termofixed at 140º C through an hour in order to establish physical interaction between solution and mesh.

A simple test to verify the process consists in weigh mesh before and after functionalization. As a result of termofixation, a gain of weight about 20% of mass must be observed. Lower gains of mass might indicate that functionalization process has been failed in proceeding.

After termofixation, mesh was washed in water in order to eliminate any residual agent and neutralization was carried out with a solution of sodium carbonate. Then, the mesh was ready to be electrospinned with its relative membrane.

Membranes seemed to be properly attached into the mesh. However, some mechanical test should have carried out in order to corroborate the success of the attachment. For that reason, this part was not included in the final report.
2.3 Monitoring of degradation by UV spectrophotometry

UV spectrophotometer uses light in the near-UV and near infrared [NIR]) ranges within the electromagnetic spectrum. It measures the absorption spectroscopy of a sample in the different wave lengths. It is a widely used tool in order to know the composition of a solution.

We proceed to carry out a motoring plan of the degradation of CHT-PEO in warm water at a temperature of 37°C. From this method, the composition of the water medium where membranes are exposed is compared to a pure water medium throughout the time in order to watch the differences. If there are no differences between both, it means that nothing is degraded from the membrane.

Three different samples of both membranes (thermal fixed membrane and membrane with genipin) were taken to do the study. The monitoring time selected was concerned in a taking of measures of absorbance after: 1h, 2h, 3 h, 24h, 2 days, 1 week and 2, 3, 6 weeks. The water was replaced after each period of time. In order to avoid any risk of contamination between both types of cross-linking two pipettes were used to extract the medium, one for each type.
Although it seems that each graph has special peculiarities, there are some aspects in common in all graphs.

In several graphs, it appears a maximum local peak between the wavelengths 270-300. It can be attributed to the degradation of chitosan into the medium.

It can be observed that after 15 minutes it tends to be the highest rate of absorbance. It means that there is a high degradation of membranes throughout the first 15 minutes of exposure into the medium. This fact coincides with what it has already been observed in the study of degradation in PBS medium, where a high loss of mass appeared after the first day of exposure in the medium. With the study of degradation it can be corroborated that in fact this high degradation do not appears after the first day but in the first minutes of exposure in medium. In fact, as it was commented before, this high degradation is due to that there is a percentage of solution which does not react. By exposing them into an aqueous medium they are removed from the membrane giving the sensation that there exists a high degradation which in fact corresponds in a removal of remainders.

Regarding the study of degradation in PBS, a higher degradation of chitosan membrane with genipin has been observed, especially throughout the first day of exposure. In the figure below it is represented the absorbance of both types of membranes after 15 minutes. Although it might be observed a higher rate of absorbance in genipin (which it would correspond to a high degradation), it is only observable in the thirt sample of genipin.
Accumulated absorbance 24h

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