DESIGN AND DEVELOPMENT OF CHITOSAN-COLLAGEN SCAFFOLD FROM BIO-WASTE FOR LIVER CANCER CELL LINE (Hep G2) ANALYSIS

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Chitosan, Collagen, Freeze drying, scaffold, yeast

Introduction:

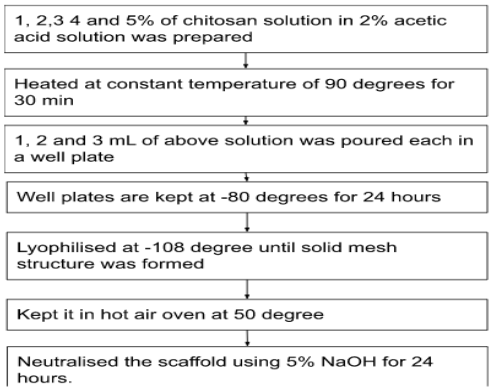
A bio-scaffold is a structure that is utilized to replace an organ permanently or temporarily, in order to restore functionality. We have designed a bio scaffold using Chitosan, which is coated with collagen. Chitosan has antimicrobial properties and collagen has very good cell adhesion properties. We have been able to extract collagen from bio waste like fish waste and characterise it. The obtained scaffold has good activity against both gram positive and gram-negative bacteria with the highest inhibition rate. Chitosan scaffolds are further stored in Deep-Freezer for removal of water and in order to increase its strength, the scaffold was freeze dried using a lyophilizer. Treatment with alkali will further improve the strength and stability of freeze-dried 3D chitosan scaffolds. We are currently carrying out scaffold characterization studies using SEM, XRD & FTIR. We are also working on carrying out yeast cell seeding on the scaffolds, as hepG2 cell lines failed to establish due to contamination and reasons unknown. Lastly, we would like to carry out few assays on the composite scaffold and conduct yeast growth related studies.

Objectives:

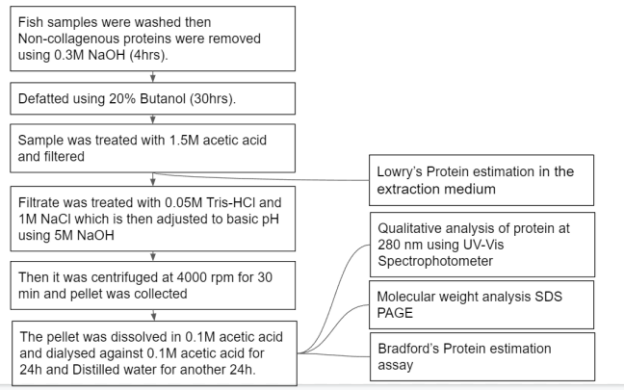
1. Developing and Characterization of Chitosan Scaffolds Through Freeze Drying Technique. Different concentrations of chitosan solutions were prepared in acetic acid. Scaffolds were lyophilized and were further treated with alkali.
2. Extraction and Characterization of collagen from Fish Skin. Pre-treatment and extraction were carried out using Fish waste. Characterization using Lowry's method, Bradford Assay and SDS PAGE.
3. Preparation of Yeast Culture (Saccharomyces cerevisiae) YPD media was prepared along with stock culture and mother culture Preparation.

Methodology:

I. Preparation of Chitosan Scaffolds



II. Extraction and Characterisation of Collagen



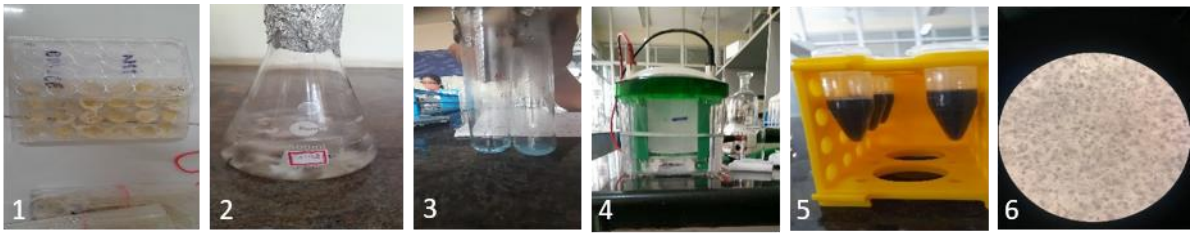
III. Preparation of Yeast media and culture

Constituents of Yeast Peptone Dextrose (YPD): Broth: Yeast extract 10g + Peptone 20g + Dextrose 20g; Media: Yeast extract 10g + Peptone 20g + Dextrose 20g + Agar-Agar 20g.

Stock Culture Preparation: 100 mL of YPD broth and 5g of dry yeast incubate for 24 hours at 37 degree in a shaker.

Mother Culture Preparation: 5mL of stock culture in 100 mL of YPD broth incubate for 24 hours at 37 degree in a shaker.

Results and Conclusions:



1. Scaffolds of different chitosan concentration of 1,2,3,4,5 % obtained as shown in Picture 1
2. Collagen Extraction and pre treatment was carried out as shown in Picture 2
3. Collagen characterisation was done using Bradford assay as shown in picture 3
4. SDS PAGE was conducted using pre stained protein ladder as shown in picture 4
5. Protein estimation using Lowry's method as shown in picture 5
6. hepG2 cells were passaged once but failed to stick to the plate and died as shown in picture 6

Scope for Future Work:

Scaffold Characterization studies: FTIR Analysis, SEM & XRD
Yeast growth assay related Studies: Anti-Microbial Assay, Swelling and Degradation Studies and Yeast growth and Viability Estimation