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Invitro Biocompatibility Assessment Of A Novel Chitosan Based Composite Membrane

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Abstract:
Objective of the study was to fabricate, characterize, and to assess the in vitro biocompatibility of chitosan-fish derived hydroxyapatite-titanium oxide nanoparticles composite membrane intended for guided bone regeneration.Then we compared the degradation rate, cytotoxicity,cell attachment, biocompatibility of chitosan membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles with commercially available collagen membrane which was the control group. The study concluded that chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide nanoparticles showed superior biocompatibility as compared to commercially available collagen membrane in all the in vitro experiments except for cell attachment on day 3 and hence can be an effective biomaterial for bone regeneration.

Background*: Chitosan is a natural guided bone regeneration membrane as it is synthesized from crustacean shells like shrimps,crabs and lobsters.However it is not an ideal material for bone regeneration, and its osteoconductivity needs to be improved.*

Materials and Methods*: Study design is an invitro comparative study.Objective was to fabricate, characterize, and to assess the in vitro biocompatibility of chitosan-fish derived hydroxyapatite-titanium oxide nanoparticles composite membrane intended for guided bone regeneration.Then we compared the degradation rate, cytotoxicity,cell attachment, biocompatibility of chitosan membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles with commercially available collagen membrane which was the control group.Fabrication of membrane was done by freeze dried lyophilization method.Morphological characterization of membrane was done using scanning electron microscope , x ray diffraction, attenuated total reflectance - fourier transform infrared spectroscopy. The membrane was subjected to degradation study tests and percentage of degradation rate was calculated in terms of weight loss.Further,in vitro cytotoxicity analysis of membrane was carried out using dimethyl thiazol tetrazolium assay in which percentage of viability of cells and dead cells were calculated.In vitro cell attachment was assessed after attaching cells to membrane and was viewed under inverted fluorescent microscope and cells were counted using image J software.Sample size was 10 for each group. Stastical analysis was done by using Shapiro Wilk test to check the normality of the data and Mann Whitney U test was used to compare between the control group and study group. All experiments were performed independently and the data was represented as mean ± standard deviation with a p value of less than 0.05 being statistically significant using Graph Pad prism.*

Results*: The results showed that chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide nanoparticles had superior compatibility when compared to commercially available collagen membrane and showed statistically significant difference among 2 groups in all the in vitro experiments except for cell attachment on day 3 which showed that result was not statistically significant.*

Conclusion: *The study concluded that chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide nanoparticles showed superior biocompatibility as compared to commercially available collagen membrane in all the in vitro experiments except for cell attachment on day 3 and hence can be an effective biomaterial for bone regeneration.*

Key Word*: Chitosan; Titanium oxide;Fish derived hydroxyapatite; Guided bone regeneration membrane*

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I. Introduction

Chitosan (CHT) {n [N-acetyl-D-glucosamine] and β-(1→4)-linked-D-glucosamine} is a natural GBR membrane as it is synthesized from crustacean shells like shrimps,crabs and lobsters and is obtained by partial deacetylation of chitin[1].It also presents excellent biological properties, like biodegradability, biocompatibility, and immunogenicity, as well as antibacterial, antifungal and wound-healing activity[2]. Its degradation products are non-toxic, nonantigenic, non-immunogenic and also non-carcinogenic. CHT has also shown to evoke minimal foreign body reaction[3]. However, CHT is not an ideal material for bone regeneration, and its osteoconductivity

needs to be improved.Also,chitosan has shortcomings like missing of cell signaling molecules ,lack of mechanical strength,fast degradation of scaffold which is required for growth of damaged tissue[4].

Lahiji et al. found that CHT may serve as an effective template for repairing osseous defects, because it has unique properties and the ability to support viable and also the functioning human osteoblasts. Thus CHT is an attractive candidate for future use in bone regeneration membranes[5]. Recent studies have highlighted that for improving the bioactivity and biocompatibility of chitosan membrane, it is necessary to combine it with other bioactive materials[6]

Titanium dioxide [TiO_2] nanoparticles addition to chitosan scaffold improved bone regeneration capability,biomineralization and sponge robustness of the scaffold,makes the scaffold highly porous which is useful in bone tissue engineering,they support cell adhesion and proliferation without producing toxicity,provide antibacterial property[7].Fish derived hydroxyapatite incorporation into chitosan membrane resulted in osteoconductive and bioactive properties,also it was biocompatible,bioactive,non toxic,non inflammatory and non immunogenic,also hydroxyapatite can accelerate bone like apatite formation on surface of implant and has chemical similarity to bone[8].

So in this study, we fabricated a chitosan based composite membrane loaded with hydroxyapatite and titanium oxide nanoparticles which could potentially be used as a guided bone regeneration membrane with the possibility to induce bone regeneration.

(10)

II. Material And Methods

For Membrane

1 weight [wt] % chitosan,1 volume [vol] % aqueous acetic acid,2% aqueous sodium hydroxide [NaOH],0.43 wt% fish derived hydroxyapatite,0.25 wt% TiO_2 nanoparticles,Polystyrene molds,Ethylene oxide.

For Degradation Rate

Chitosan-TiO_2-fish derived hydroxyapatite composite membrane,Commercially available,collagen membrane [Healiguide], Simulated body fluid [SBF]

For Cell Culture

Saos-2 [Sarcoma osteogenic] osteoblast cells, Dulbecco's Eagle's medium, Bovine serum, Sodium bi carbonate, Sodium pyruvate, Penicillin, Streptomycin, Amphotericin B, Haematoxylin and Eosin staining.

For Cytotoxicity

Chitosan based membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles, Commercially available collagen membrane [Healiguide], Dulbecco's Modified Eagle's medium,10% Foetal bovine serum [FBS], Saos-2 osteoblast cells, Cisplatin or 30% ethanol,96 well plates,10 % MTT [Dimethyl Thiazol Tetrazolium] reagent, MTT lysis buffer [DMSO] Dimethyl sulfoxide, ELISA microplate reader

For Cell Attachment Assay

Saos-2 osteoblast cells,Dulbecco's Modified Eagle's Medium (DMEM),10% fetal bovine serum,Streptomycin,Penicillin,Inverted fluorescent microscope,Image J software.

Study Design: An vitro comparative study
Study Location: Pushpagiri research center,pushpagiri dental college.
Study Duration: May 2019 to June 2022.
Sample size: 10
Sample size calculation: Assuming the prevalence of nontoxicity of 90% with power 80% and 95% confidence interval [68], the required minimum sample size was 10 using the formula:

$$n = \frac{Z^2_{\left(1-\frac{\alpha}{2}\right)} P(1-P)}{d^2}$$

where, Z is the confidence interval P is the prevalence d is the power

Inclusion criteria:

Commercially available collagen membrane [Healiguide] and chitosan membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles.

Exclusion criteria:
Chitosan membrane with high water content.

Procedure methodology
1] Fabrication of chitosan based membrane loaded with hydroxyapatite and TiO_2 nanoparticles
2] Morphology characterization
3] Cell culture in vitro
4] Cytotoxicity testing of scaffold in vitro
5] Cell attachment in vitro
6] Biocompatibility in vitro
7] Comparative evaluation of chitosan based membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles with commercially available collagen membrane

Statistical analysis
Degradation Rate

All experiments were performed independently and the data was represented as mean ± standard deviation (SD) with a p value of less than 0.05 being statistically significant using Graph Pad prism.Shapiro Wilk test was used to check the normality of the data and it revealed that the data was not normal.And thus,Non parametric tests were used for the analysis.Mann Whitney U test was used to compare between the control group and study group.

MTT ASSAY

All experiments were performed independently and the data was represented as mean ± standard deviation (SD) with a p value of less than 0.05 being statistically significant using Graph Pad prism. Shapiro Wilk test was used to check the normality of the data and it revealed that the data was not normal.And thus,Non parametric tests were used for the analysis. Mann Whitney U test was used to compare between the control group and study group.

Invitro Cell Attachment

All experiments were performed independently and the data was represented as mean ± standard deviation (SD) with a p value of less than 0.05 being statistically significant using Graph Pad prism. Shapiro Wilk test was used to check the normality of the data and it revealed that the data was not normal.And thus,Non parametric tests were used for the analysis. Mann Whitney U test was used to compare between the control group and study group.

III. Result

Scanning Electron Microscopy

SEM images of chitosan based membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles is shown in Figure 1 and indicate a porous structure. This composite membrane had a more homogeneous pore-size distribution and displayed penetrating inter-connecting pores both on the surface and in the cross-sectional view The [Figure 1b] clearly exhibited the intercalation of thin TiO_2 (red arrow) in the chitosan/hydroxyapatite interfaces shown in higher magnification at 1000X. The presence of spherical particles with different sizes on the surface of samples was observed. Consequently, a smaller amount of particles is formed known to be HA particles (green arrow) uniformly mixed with chitosan shown in [Figure 1a] at lower magnification 500X. At higher magnification 10000X the SEM images shows uniform smooth and spongy surface of chitosan, shown in [Figure 1d].

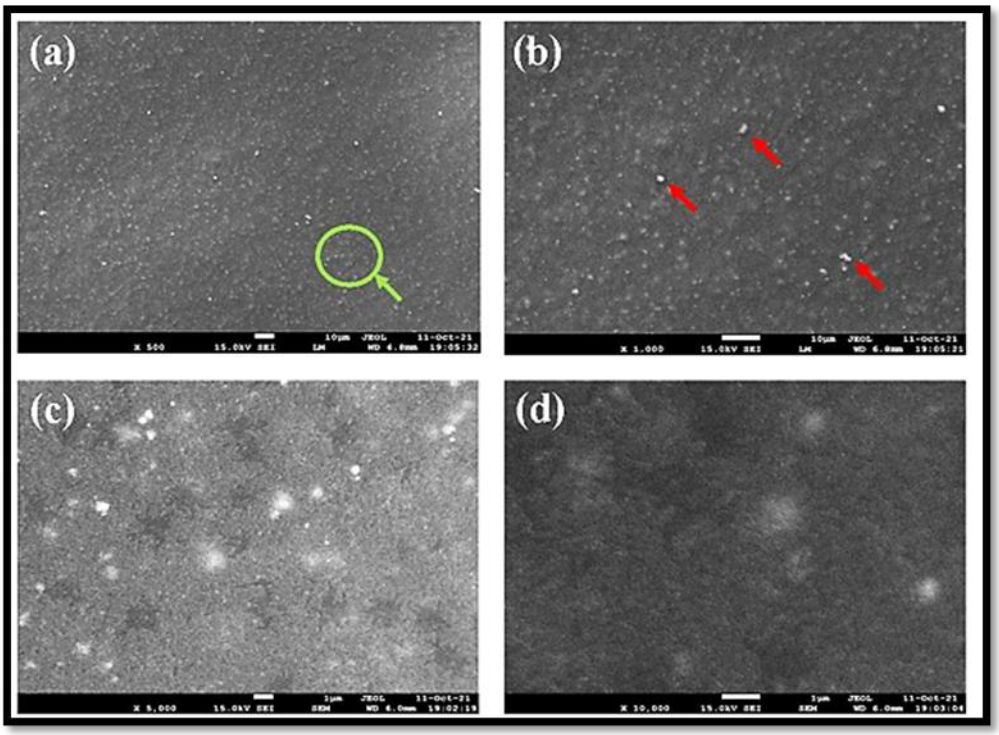


Figure 1: Scanning electron microscope showing images of chitosan incorporated with hydroxyapatite and TiO2 at different magnifications

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy [FTIR-ATR]

FTIR-ATR of the chitosan based membrane loaded with fish derived hydroxyapatite and TiO_2 nanoparticles was performed for the analysis of the functional group of proteins. The FTIR spectra of the composite membrane is shown in the figure 2. When the wave patterns were compared, some major peaks were noticed. The broad bands at 400 to 800 cm^{-1} could be ascribed to the bending vibration Ti-O-Ti and Ti-O bonds in the anatase lattice. The observed peaks centered at around 3550 - 3650 cm^{-1} could be attributed to stretching modes of surface-adsorbed water (-OH). The spectrum for the nanoporous TiO also presents bands at 2923 and 2841 cm^{-1}, assigned to ν(CH) vibrations of residual organic compounds, and some small bands in the 1000-1555 cm^{-1} range, probably originated by stretching vibration of carbonates C=O. The bands centered at 551 cm^{-1}, 601 cm^{-1}, 1085 cm^{-1}, 1009 cm^{-1},959 cm^{-1}, can be ascribed to vibration modes of PO_4^{3-}. Their intensity confirmed the presence of hydroxyapatite in the chitosan TiO_2 composite.

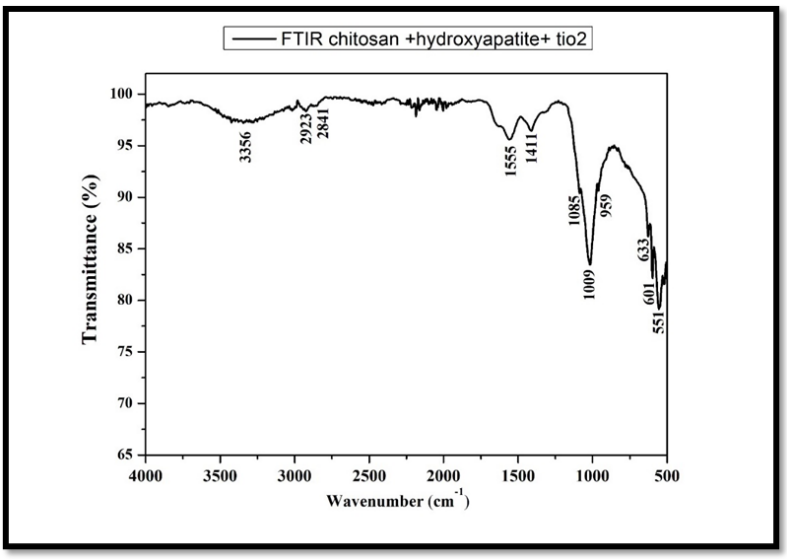


Figure 2 : FTIR-ATR of the chitosan based membrane loaded with fish derived hydroxyapatite and TiO2 nanoparticles

X-RAY Diffraction [XRD]

The X-ray diffraction (XRD) was used analyze the crystalline nature of the prepared chitosan hydroxyapatite titanium oxide composite. Figure 3 shows broad XRD diffraction peak of chitosan at 2θ = 26°corresponds to (130) planes, emphasized the amorphous nature of chitosan [JCPDS No. 39-1894]. The hydroxyapatite (HA) showed the highest intensity peak at 32.19° and 32.9° of 2θ corresponded to (112) and (300) plane, which matched to the key peak of hexagonal HA structure. The XRD pattern of HA exhibited the sharp peaks at all 2θ, and the significant clear peaks at 2θ range of 32.19° and 32.9°, which indicated the perfect crystalline structure of HA sample. A single phase diffraction pattern was identified for HA in accordance to the [JCPDF 9-432]. The XRD pattern of TiO_2 agrees with the JCPDS card no. 21-1272 of (anatase) phase. The 2θ peak at 38.8° confirmed the TiO_2 anatase structure.

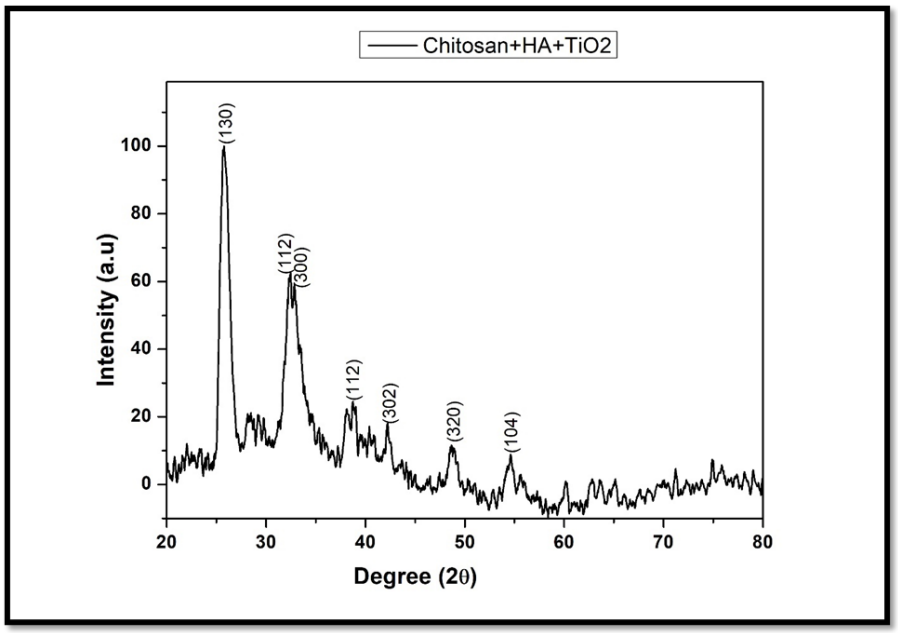


Figure 3: X-ray diffraction (XRD) of chitosan- hydroxyapatite - titanium oxide composite membrane

Degradation Rate

Material dissolution was evaluated in terms of weight loss. After 2,4,6,8 weeks , both the control group and study group, scaffolds were removed from the fluid , rinsed in distilled water and was dried in an oven for 12 hours.Weight of the membrane was measured in weighing machine.Weight reading of weighing machine was converted into milligrams.

% of weight loss were computed according to the following equation:
$Wt\ \% = 100 \times (W_o - W_t)/W_o$
where ; W_o is initial weight of the sample and W_t is dry weight of the sample at time t

For Control Group
Initial weight of membrane was 98.5 mg
Weight of membrane at 2 weeks was 85.3 mg
Weight of membrane at 4 weeks was 79.2 mg
Weight of membrane at 6 weeks was 61.1 mg
Weight of membrane at 8 weeks was 59.8 mg

For Study Group
Initial weight of membrane was 141.3 mg
Weight of membrane at 2 weeks was 132.1 mg
Weight of membrane at 4 weeks was 127.0 mg
Weight of membrane at 6 weeks was 113.2 mg
Weight of membrane at 8 weeks was 106.1 mg

Percentage of weight loss using the formula
$Wt\ \% = 100 \times (W_o - W_t)/W_o$;

where ; W_o is initial weight of the sample and W_t is dry weight of the sample at time t

Percentage [%] Of Weight Loss For Control Group [Collagen Membrane – Healiguide]
% of weight loss at 2 weeks was 13.40 %
% of weight loss at 4 weeks was 19.59 %
% of weight loss at 6 weeks was 37.96 %
% of weight loss at 8 weeks was 39.28 %

Percentage [%] Of Weight Loss For Study Group [Chitosan -Fish Derved Hydroxyapatite - Titanium Dioxide Nanoparticles]
% of weight loss at 2 weeks was 6.51 %
% of weight loss at 4 weeks was 10.12 %
% of weight loss at 6 weeks was 19.88 %
% of weight loss at 8 weeks was 24.91 %

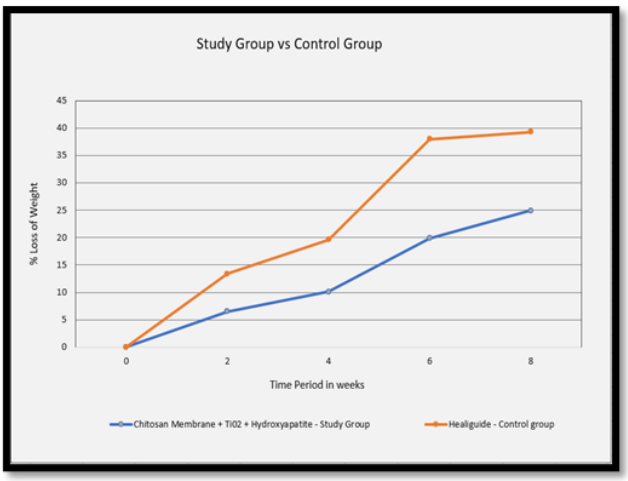
SAMPLE	DURATION	INITIAL WEIGHT	FINAL WEIGHT	% OF WEIGHT LOSS
CONTROL GROUP	2 WEEKS	98.5 mg	85.3 mg	13.40 %
	4 WEEKS	98.5 mg	79.2 mg	19.59%
	6 WEEKS	98.5 mg	61.1 mg	37.96%
	8 WEEKS	98.5 mg	59.8 mg	39.28%
STUDY GROUP	2 WEEKS	141.3 mg	132.1 mg	6.51%
	4 WEEKS	141.3 mg	127.0 mg	10.12%
	6 WEEKS	141.3 mg	113.2 mg	19.88%

The mean % of weight loss of 10 samples in the control group at 2 weeks was 12.06 with a standard deviation [SD] of 1.84 whereas for the study group , % of weight loss was found to be 5.64 with a SD of 1.45.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was less than 0.001 which showed that there was statistically significant difference in the mean values of % weight loss among 2 groups.

The mean % of weight loss of 10 samples in the control group at 4 weeks was 20.17 with a SD of 1.41 whereas for the study group , % of weight loss was found to be 10.07 with a SD of 0.10.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was less than 0.001 which showed that there was statistically significant difference in the mean values of % weight loss among 2 groups.

The mean % of weight loss of 10 samples in the control group at 6 weeks was 37.81 with a SD of 0.75 whereas for the study group , % of weight loss was found to be 19.88 with a SD of 0.27.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was less than 0.001 which showed that there was statistically significant difference in the mean values of % weight loss among 2 groups.

The mean % of weight loss of 10 samples in the control group at 8 weeks was 39.04 with a SD of 0.71 whereas for the study group , % of weight loss was found to be 24.88 with a SD of 0.35.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was less than 0.001 which showed that there was statistically significant difference in the mean values of % weight loss among 2 groups



MTT ASSAY (Dimethyl Thiazol Tetrazolium Assay)

The invitro biocompatibility and proliferation of cells on chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide particles was assessed by MTT assay.

Among 2 different groups when 10 samples were tested for percentage of viable cells and dead cells and values obtained are shown in table 10 for control group and table 11 for study group and mean of values of 10 samples were calculated for percentage of viable cells and dead cells.MTT assay of chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide particles at 24 hours showed maximum cell viability of 97.337% .Whereas at 24 hours ,healiguide control group showed cell viability 94.774% only. Analyses of study group was also done at 48 hours and 72 hours and found that composite membrane showed 98.651% at 48 hours and 99.490% at 72 hours. Whereas healiguide control group showed cell viability of 96.185% only at 48 hours and cell viability of 97.149% only at 72 hours.

Percentage of dead cells were : Control group showed dead cells % at 24 hours as 5.225% ; whereas study group showed dead cell % at 24 hours as 2.662% and shown in Control group showed dead cells % at 48 hours as 3.814% ; whereas study group showed dead cell % at 48 hours as 1.351% in Control group showed dead cells % at 72 hours as 2.851%; whereas study group showed dead cell % at 72 hours as 0.509 .

The mean % of viable cells of 10 samples in control group at **24 hours** was found to be 94.774 with SD 1.72, whereas for the study group , the mean % of viable cells at 24 hours was found to be 97.337 with SD of 1.69.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.010 which shows statistically significant difference in mean % of viable cells between control and study group.

The mean % of viable cells of 10 samples in control group at **48 hours** was found to be 96.185 with SD 1.72, whereas for the study group , the mean % of viable cells at 48 hours was found to be 98.651 with SD of 0.92.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.004 which shows statistically significant difference in mean % of viable cells between control and study group.

The mean % of viable cells of 10 samples in control group at **72 hours** was found to be 97.149 with SD 1.32, whereas for the study group , the mean % of viable cells at 72 hours was found to be 99.490 with SD of 0.52.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.001 which shows statistically significant difference in mean % of viable cells between control and study group.

The mean % of dead cells of 10 samples in control group at **24 hours** was found to be 5.225 with SD 1.72, whereas for the study group , the mean % of dead cells at 24 hours was found to be 2.662 with SD of 1.69.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.010 which shows statistically significant difference in mean % of dead cells between control and study group.

The mean % of dead cells of 10 samples in control group at **48 hours** was found to be 3.814 with SD 1.72, whereas for the study group , the mean % of dead cells at 48 hours was found to be 1.315 with SD of 0.92.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.004 which shows statistically significant difference in mean % of dead cells between control and study group.

The mean % of dead cells of 10 samples in control group at **72 hours** was found to be 2.851 with SD 1.32, whereas for the study group , the mean % of dead cells at 48 hours was found to be 0.509 with SD of 0.52.Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.001 which shows statistically significant difference in mean % of dead cells between control and study group.

Cell Viability Percentage Of 10 Samples In Control Group Using Mtt Assay And Dead Cell Percentage Shown

SAMPLE	24 HOURS		48 HOURS		72 HOURS	
	% Viable Cells	% Dead Cells	% Viable Cells	% Dead Cells	% Viable Cells	% Dead Cells
SM1	94.654	5.346	96.224	3.776	97.223	2.777
SM2	93.028	6.972	94.012	5.988	95.654	4.346
SM3	92.126	7.874	94.118	5.882	95.612	4.388
SM4	94.323	5.677	95.672	4.328	96.722	3.278
SM5	95.826	4.174	94.892	5.108	95.821	4.179
SM6	93.027	6.973	97.012	2.988	97.924	2.076
SM7	97.886	2.114	99.112	0.888	99.252	0.748
SM8	96.257	3.743	95.228	4.772	96.422	3.578
SM9	95.386	4.614	97.382	2.618	97.916	2.084
SM10	95.228	4.772	98.201	1.799	98.944	1.056
MEAN ± SD	94.774 ± 1.72	5.225 ± 1.72	96.185 ± 1.72	3.814 ± 1.72	97.149 ± 1.32	2.851 ± 1.32

Cell Viability Percentage Of Of 10 Samples In Study Group Using Mtt Assay And Dead Cell Percentage Shown

SAMPLE	24 HOURS		48 HOURS		72 HOURS	
	% Viable Cells	% Dead Cells	% Viable Cells	% Dead Cells	% Viable Cells	% Dead Cells
SM1	95.227	4.773	99.112	0.888	99.524	0.476
SM2	96.257	3.743	98.201	1.799	99.622	0.378
SM3	95.386	4.614	97.382	2.618	98.312	1.688
SM4	97.886	2.114	99.442	0.558	99.952	0.048
SM5	98.432	1.568	99.682	0.318	99.824	0.176
SM6	95.228	4.772	99.026	0.974	99.714	0.286
SM7	99.112	0.888	99.251	0.749	99.626	0.374
SM8	99.442	0.558	96.851	3.149	98.814	1.186
SM9	97.382	2.618	98.522	1.478	99.656	0.344
SM10	99.026	0.974	99.012	0.988	99.857	0.143
MEAN ± SD	97.337± 1.69	2.662 ± 1.69	98.651± 0.92	1.351 ± 0.92	99.490 ± 0.52	0.509 ± 0.52

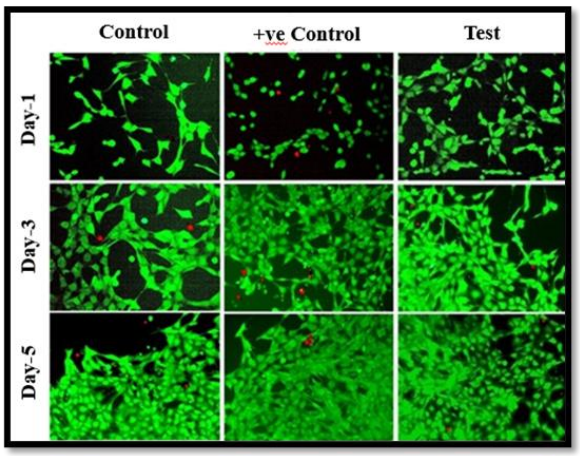
Invitro Cell Attachement

Cell attachment on the membrane were determined on days 1, 3, and 5 and the results are shown in Figure. The cells seeded wells in 96 plates were used as controls for comparison. The values of attached cells in positive control group and study group for 10 samples are shown in for control group and for study group.It was observed that the mean values in positive control and test group at day 1, that osteoblasts could only adhere on the surface of the samples covering approximately 14 to 22 % in positive control group and 16-26 % in study group/test group. This was due to the low initial cell seeding density in order to observe proliferation over a week span. The cells showed their characteristic spindle shaped morphology with good attachments to surface of membrane. Afterward, at day 3, cell density increased and reached about approximately 32 to 42% in positive control group and 36 to 44% in study/test group. Osteoblast proliferation and division continued gradually in all samples irrespective of the test group, reaching a confluency of about 70 to 80% in positive control group and 74 to 82 % in study/test group. Both positive control and test group scaffolds were almost completely covered with a continuous layer of osteoblast cells. The cells would continue to proliferate until confluence, when there would be no more available surface area on the constructs for further proliferation.

The mean % of attached cells of 10 samples in control group on day 1 was found to be 18.40 with SD 2.63 whereas for the study group , mean % of attached cells was found to be 21.40 with SD 2.98. Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.034 which showed statistically significant difference in mean % of attached cells between control and study group.

The mean % of attached cells of 10 samples in control group on day 3 was found to be 37.20 with SD 4.23 whereas for the study group , mean % of attached cells was found to be 40.40 with SD 2.79. Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.082 which showed that the mean % of attached cells between control and study group on day 3 was not statistically significant.

The mean % of attached cells of 10 samples in control group on day 5 was found to be 73.00 with SD 3.01 whereas for the study group , mean % of attached cells was found to be 76.60 with SD 2.67. Mann Whitney U test was used to compare between control and study group for statistical analysis and it was found that the p value was 0.007 which showed statistically significant difference in mean % of attached cells between control and study group.



Cell Attachment Percentage Of 10 Samples In Control Group

SAMPLE	DAY 1	DAY 3	DAY 5
SM1	20%	40%	72%
SM2	22%	42%	80%
SM3	18%	36%	72%
SM4	16%	32%	70%
SM5	22%	36%	72%
SM6	20%	42%	70%
SM7	14%	32%	72%
SM8	18%	42%	74%
SM9	18%	38%	76%
SM10	16%	32%	72%
MEAN ± SD	18.40 ± 2.63	37.20 ± 4.23	73.00 ± 3.01

Cell Attachment Percentage Of 10 Samples In Study Group

SAMPLE	DAY 1	DAY 3	DAY 5
SM1	22%	42%	78%
SM2	24%	44%	82%
SM3	20%	40%	76%
SM4	18%	38%	74%
SM5	26%	38%	76%
SM6	22%	44%	74%
SM7	16%	36%	76%
SM8	20%	42%	74%
SM9	22%	42%	80%
SM10	24%	38%	76%
MEAN ± SD	21.40 ± 2.98	40.40 ± 2.79	76.60 ± 2.67

IV. Discussion

In the present study, we attempted to fabricate chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide nanoparticles for guided bone regeneration and was prepared by freeze dried lyophilization method and was carried out in CIFT , Central Institute of Fisheries and Technology.

Every year, enormous amount of fish waste is being discarded ,hence finding adequate modalities for converting these marine waste residues into useful products of high significance and economic value is of essence. Since fish scale derived hydroxyapatite and chitosan are a derivative of marine waste, it is a cost effective approach. Hence, regenerative procedures are easily available to all strata of the society. The unique design, chemistry and functionality makes them a highly demanded option in bone regeneration.

For the characterisation of membrane , SEM , FTIR and XRD analyses were done. SEM analysis confirmed three dimensional porous structure and excellent pore interconnectivity of the membrane and FTIR analysis confirmed the functional group of proteins in the membrane and XRD confirmed structure of crystals in the membrane . Degradation studies proved that the study group membrane degraded at a slower rate when compared to commercially available collagen membrane due to the presence of titanium oxide nanoparticles. In vitro biocompatibility assessment using MTT assay proved that the material was not cytotoxic and was biocompatible. In vitro cell attachment studies showed that osteoblast cells could adhere more on to the surface of the study group membrane when compared to collagen membrane due to the presence of fish scale derived hydroxyapatite and titanium oxide nanoparticles.

Overall, the results of the present study suggested that chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide nanoparticles can be a promising substitute for guided bone regeneration. However, future studies are needed to validate the findings and to clarify the translation potential of this marine biomaterial in to a clinical setting.

V. Conclusion

The results of the study suggested that chitosan membrane loaded with fish derived hydroxyapatite and titanium oxide nanoparticles can be a promising substitute for guided bone regeneration. However, future studies are needed to validate the findings and to clarify the translation potential of this marine biomaterial in to a clinical setting.

References

[1]. Elgali I, Omar O, Dahlin C, Thomsen P. Guided Bone Regeneration: Materials And Biological Mechanisms Revisited Eur J Oral Sci 2017; 125: 315–337. © 2017

[2]. Ezoddini-Ardakani F, Azam A.N, Yassaei S, Fatehi F, Rouhi G. Effects Of Chitosan On Dental Bone Repair. Health 2011; 3(5)200-205.

[3]. Alves NM, Mano JF. Chitosan Derivatives Obtained By Chemical Modifications For Biomedical And Environmental Applications. Int J Biol Macromol 2008;43:401–14.

[4]. Lahiji A, Sohrabi A, Hungerford DS, Frondoza CG. Chitosan Supports The Expression Of Extracellular Matrix Proteins In Human Osteoblasts And Chondrocytes. J Biomed Mater Res 2000;51:586–95.
[5]. Mota J, Yu N, Caridade SG, Luz GM, Gomes ME, Reis RL, Jansen JA, Walboomers XF, Mano JF. Chitosan/Bioactive Glass Nanoparticle Composite Membranes For Periodontal Regeneration. Acta Biomaterialia. 2012 Nov 1;8(11):4173-80.
[6]. Ikono R, Li N, Pratama NH, Vibriani A, Retnoyuniarni D, Luthfansyah M, Bachtiar BM, Bachtiar E, Nasikin M, Kagami H, Li X. Enhanced Bone Regeneration Capability Of Chitosan Sponge Coated With Tio2 Nanoparticles. Biotechnology Reports. 2019 Jun 5:E00350.
[7]. Itoh S, Kikuchi M, Takakuda K, Koyama Y, Matsumoto HN, Ichinose S, Tanaka J, Kawauchi T, Shinomiya K. The Biocompatibility And Osteoconductive Activity Of A Novel Hydroxyapatite/Collagen Composite Biomaterial, And Its Function As A Carrier Of Rhbmp-2. Journal Of Biomedical Materials Research: An Official Journal Of The Society For Biomaterials And The Japanese Society For Biomaterials. 2001 Mar 5;54(3):445-53.
[8]. Behra SS , Das U , Bissoyi A , Singh AK. Chitosan / Tio2 Composite Membrane Improves Proliferation And Survival Of L929 Fibroblast Cells:Application In Wound Dressing And Skin Regeneration.International Journal Of Biological Macromolecules.2017 May 1;98:329-40.
[9]. Maachoua H,Bal KE,Bal Y,Chagnes A,Cote G, Alliouche D.Characterization And In Vitro Bioactivity Of Chitosan/Hydroxyapatite Composite Membrane Prepared By Freeze – Gelation Method.Trends In Biomaterials And Artificial Organs:22(1)16-27;May 2008.