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Research Article

Evaluation of Hydrophilic Gel Made from Aloe Vera and Curcuminoids for Metabolism of Osteoblasts on Titanium Discs-*In Vitro* Study

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Abstract

Aims and Objectives: The aim of the study is to evaluate the effect of hydrophilic gel made from aloe Vera and curcuminoids, this study looked at the direct long-term effect of hydrophilic gel on different cell lines. The objective is to assess Cell viability, differentiation and morphology as well as pH and calcium uptake to assess the overall biocompatibility of the tested materials.

Materials and Methods: The hydro gel was formulated in to 3 groups. Sample 1 had 75% acemannan and 25% curcuminoids sample 2 had both 50% in equal quantities sample 3 had 25% aloe Vera and 75% curcuminoids. The dimension of the titanium disc on which the gel was coated was 5 mm in diameter and 2 mm in width. MTT ASSAY is done to evaluate the cell viability, Materials used for MTT Assay are MTT Powder, DMSO, CO_2 incubator Plate rear, Centrifuge, cell lines used in this study was MG 63(Homo sapiens bone Osteosarcoma) Cell line which was procured from National center for cell science Pune India.

Results: Previous studies have shown higher values for cell viability measured by MTT test compared with the present study, turmeric which has excellent anti-inflammatory and bone healing properties were added as a second ingredient to the gel. Osteoblastic differentiation with sample 1 at the end of 24 hrs was 0.0837 over all % cell inhibition was 75.67, with sample 2 at the end of 24 hrs it was 0.2402 % cell inhibition over all was 57.67% for sample 3 at the end of 24 hrs 0.1643 overall was 75.62%.

Conclusion: The proliferation of cells was markedly inhibited upon exposure of cells to the gel. Curcumin did not induce apoptosis but arrested cells At G1 phase of cell cycle.

In addition, cur cumin stimulated the expression of mRNA for P21, which inhibits the activity of cyclin dependent kinases and inhibited the phosphorylation of histone H1.

Furthermore, the gel reduced the rate of deposition of calcium and formation of mineralized nodules.

Our results indicated that the formulated gel might inhibit proliferation and mineralization of osteoblastic cells through expression of P21.

Keywords: *Hydrophilic Gel; Aloe Vera; Curcuminoids; Osteoblasts; Titanium*

Introduction

Enhancing dental implant Osseointegration has been a area of research for more than 4 decades now. Researcher have tried from macro geometry to micro geometry, change in materials, drill sequences etc to enhance Osseointegration, as it improves the success rate of the implant restoration [1]. It is well acknowledged that the quality and quantity of host bone, presence of sufficient primary stability at the time of implant placement and formation of a direct bone-to-implant contact (BIC) are critical parameters that govern the overall success and survival of implants. However, implant surface characteristics (including surface topography, energy, chemistry, and roughness) also play significant roles in enhancing osseointegration and BIC [1-4]. Studies have reported that increasing surface roughness of implants favors osteoblastic proliferation, collagen synthesis, and expression of integrins in the extracellular matrix, thereby improving the mechanisms associated with osseointegration [5-14]. One important factor behind the good outcomes is probably the use of enhanced implant surfaces. Experimental research has shown that a slight increase of surface roughness results in a stronger bone tissue response compared with both smoother and rougher implant surfaces. It has been shown that porous titanium implants can be enriched, either directly or by association with biocompatible hydrogels, with antibiotics, VEGF, and FGF-2. To improve implant osseointegration, osteogenic factors can be used for implant enrichment [15,16].

In this regard, some studies placed localized organic and inorganic osteogenic coatings on implant surfaces in an attempt to improve implant surface activity and osteopromotive activity. Literature has shown that hydrophilicity enhances more bone to implant contact [17-19]. Hydro gels which are hydrophilic in nature and which can give an sustained release of bone proliferating medicine, can be incorporated in the gel. The aim of the present study was to compare and evaluate the cell proliferation on disc with applied with hydrophilic gel made from the acemannan, a polysaccharide extracted from aloe vera gel, as it promotes tissue healing and curcuminoids, which have the bone protective mechanism [20,21].

Materials and Methods

This study looked at the direct long-term effect of hydrophilic gel on different cell lines. Cell viability, differentiation and morphology as well as pH and calcium uptake were analysed to assess the overall biocompatibility of the tested materials. It was planned to evaluate the long-term effects of thus prepared gel on osteoblast cells to simulate the *in-vivo* situation as closely as possible. The hydrogel was formulated in to 3 groups. Sample 1 had 75% acemannan and 25% curcuminoids, sample 2 had both 50% in equal quantities, and sample 3 had 25% aloe vera and 75% curcuminoids. The dimension of the titanium disc on which the gel was coated was 5 mm in diameter and 2mm in width. Materials used for MTT Assay are MTT Powder, DMSO, CO_2 incubator, Plate rear, Centrifuge, cell lines used in this study was MG 63 (Homo sapiens bone Osteosarcoma) Cell line which was procured from National center for cell science Pune India.

Procedure

Trypsinize 70-80% confluent cell lines (MG-63), Check for the viability and centrifuge the cells. The sample 1, 2 and 3 put on each titanium disk. After half an hour, samples with titanium disks were placed in each 96-well plate. Seeded 50000 cells/well in 96 well plate and incubated for 24 hrs at 37°C, 5 % CO_2 incubator.

After 24 hrs of incubation, the media was removed and a fresh complete media was added and again kept it for incubation for another 24 hrs for proliferation of cell.

After 24 hrs, the media was removed from the wells and 100 µl/well (50 µg/well) of the MTT (5 mg/10 ml of MTT in 1X PBS, the solution is filtered through a 0.2 µm filter and stored at 2 - 8°C for frequent use or frozen for extended periods) working solution was added and incubated for next 3 to 4 hours.

After incubation with MTT reagent, the media was removed from the wells and 100 µl of DMSO was added to rapidly solubilize the formazan, and it was measured for the Absorbance at 590 nm.

% of Inhibition= 100-(Sample/Control) x 100. The Titanium discs, were coated with the hydrophilic gel made from aloe vera and curcuminoids in different combinations, by immersing the discs in gel for around 45 - 60 sec so that the contents are properly coated around the discs, and are released in a controlled manner.

Cell Viability

To determine cell viability a MTT assay was conducted. Briefly, 10,000 cells per cm2 are seeded into 12-well plates containing gel applied and non-gel applied discs and F12K medium with 20 % FCS and 100 µg/g streptomycin. The cell medium was changed once a week during the experiment. In total 9 wells per specimen and time point was tested. After 24h and 7, 14, and 21 days MTT solution was added to the cell medium. The cells were then incubated in dark for 4 h at 37°C. Subsequently the cell medium were discarded and the cells were lysed with 0.004 N HCl in isopropanol. The cell lysates were centrifuged and supernatants was transferred as triplets to a 96-well plate. The adsorption was measured at 570 and 630 nm using a Synergy HT Microplate Reader (BioTek, Bad Friedrichshall, Germany). The MTT assay was also performed for the discs which were not seeded with the cell culture in order to exclude material's effect on the test and see only how the cells react during the assay.

Additionally, cell morphology was studied by inverted light microscopy using a Leica microscope type 090-135.002 (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a Nikon Ds-Fi1 digital camera (Nikon, Duesseldorf, Germany) [22-23].

Alkaline phosphatase (ALP) content

As an indicator of changes in the differentiation behavior of the bone-forming cells caused by the test substances a SensoLyte® pNPP Alkaline Phosphatase assay (AnaSpec, Fremont, CA) was applied after 24h and 7, 14, and 21 days of culturing in Dulbecco's modified Eagle's medium (DMEM) low glucose with l-glutamine, 10 % FCS, 100 U/ml penicillin, 100 µg/g streptomycin, 0.1 µM dexamethazone, 0.005 µM ascorbic acid and 10 mM β-glycerol phosphate to induce osteogenic differentiation. The cell medium was changed once a week during the experiment. In total 9 wells per specimen and timepoint were tested.

The cells were washed and frozen at −80°C. After thawing the cell number were measured using a PicoGreen® dsDNA quantitation assay (In vitrogen, Eugene, OR) according to the manufacturer's protocol. Cells will be lysed with 1% Triton X-100 in phosphate-buffered saline. The cell lysates was centrifuged and the supernatants were mixed with the PicoGreen® working solution in a 96-well plate. The samples were excited at 485 nm and the fluorescence emission intensity measured at 528 nm. The cells lysed for the PicoGreen assay, were centrifuged and the supernatants was diluted in specific assay buffer. ALP substrate was applied to the diluted samples and the absorbance was measured at 405 nm. The absolute amounts of ALP was correlated with the cell numbers obtained from the PicoGreen® assay. Both ALP and PicoGreen assays was additionally be carried out for the discs which were not seeded with the cell culture in order to exclude material's effect on the test and see only how the cells react during the assays [23].

Transmission Electron Microscopy

Human MSC seeded in chamber slides (Nalge Nunc International, Rochester, NY) was incubated with magnesium discs for 7 and 21 days. The cell layer was fixed for 30 min with 2 % paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2 - 7.4) with 2 % glutaraldehyde and 0.02 % picric acid, followed by 20 min fixation with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2 - 7.4). The samples were dehydrated and embedded in Epon before ultrathin sections (80 - 100 nm) was applied to collodion-coated copper grids.

Analysis was done with a Leo 912 transmission electron microscope (Carl Zeiss AG, Oberkochen, Germany) at 80 kV accelerating voltage and equipped with a TRS Sharpeye slow scan dual speed CCD camera (Albert Troendle Prototypentwicklung, Moorenweis, Germany) [23].

Results

Acemannan had Good cell viability from day 1, Pure acemannan had high viability at the very first day, but then the viability decreased .Osteoblastic differentiation with sample 1 at the end of 24 hrs was 0.0837 over all % cell inhibition was 75.67, with sample 2 at the end of 24 hrs it was 0.2402% cell inhibition over all was 57.67% for sample 3 at the end of 24 hrs 0.1643 overall was 75.62%, change in concentration could not create any favourable condition for the proliferation of the cells, secondly the other condition was the use of cancerous cell line, turmeric previously showed to have tremendous effect on killing cancer cells which also might have contributed for the negative outcome of the results, studies have to be undertaken on other cell lines as well (Table 1).

Sample	OD (n = 1)	OD (n = 2)	OD (n = 3)	Average	% inhibition	Comment
(Control) Only Titanium disc	0.7288	0.6067	0.5061	0.6138	0	
Sample 1 + Titanium disc	0.0837	0.195	0.1696	0.1494	75.65	No proliferation
Sample 2 + Titanium disc	0.2402	0.3032	0.2361	0.2598	57.67	No proliferation
Sample 3 + Titanium disc	0.1643	0.1334	0.1511	0.1496	75.62	No proliferation

Table 1: *Absorbance values of effect of sample with titanium disk on MG-63 cells by MTT assay.*

Discussion

This study evaluated the long-term effects of the gel on osteoblast cells.

An important drawback of tetrazolium-based tests is that the difference between cytotoxic (cell death) and cytostatic (reduced growth rate) effects cannot be distinguished [24].

We thus looked at cell morphology under light microscopy and TEM. After examination, it was revealed that the number of cells decreased in the presence of hydrophilic gel. These materials seem to have long-term cytotoxic effects on cancer cell lines when placed in direct contact with the cells.

Degradation particles were also found in the cytoplasm. The presence of high amounts of degradation products inside the cells could explain the lower cell viability values for hydrophilic gel. It was shown in previous studies that uptake of material particles leads to induction of cell stress which triggers cytotoxicity. here in the present study, we showed that the treatment of MG-63 cells with curcumin inhibited their proliferation. This result is consistent with previous studies performed in humans [27] and rats [28] osteoblast, indicating that curcumin inhibites the proliferation of several cell types, most likely due to an apoptosis-dependent mechanism. This mechanism has not been completely elucidated, but it may be, at least in part, due to the ability of curcumin to suppress the activation of AP-1, a dimeric transcription factor consisting of a Fos-related Osteoblast differentiation comprises three distinct processes: proliferation, maturation of the extracellular matrix and mineralization. During their developmental sequence, osteoblasts express genes associated with differentiation Osteoblast differentiation comprise three distinct processes: proliferation, maturation of the extracellular matrix and mineralization. During their developmental sequence, osteoblasts express genes associated with differentiation protein and a Jun-related protein as recently described [29].

In the present study, changes were observed in the early stages, with increased ALP expression in curcumin-stimulated cells at seven days post-culture. However, there was a significant decrease in the late stages of osteoblast differentiation at day 21, evidenced by the decelerating accumulation of calcium in osteoblasts.

In previously reported studies, curcumin has been reported to cause the arrest of cell cycle and the upregulation of expression of p21WAF1/CIP1 [30-32]. Furthermore, there is a report that p21WAF1/CIP1 mediates G1 arrest [34]. Thus, the inhibitory effects of curcumin on proliferation of ROB cells might be due, in part, to the arrest of cell cycle progression via expression of p21WAF1/CIP1. In addition, curcumin inhibited the mineralization by ROB cells. Recently, p21WAF1/CIP1 has been reported to act as a brake in osteoblast differentiation by using p21WAF1/CIP1 null mice [33]. Furthermore, transient overexpression of p21WAF1/CIP1 from an adenovirus vector delayed the onset of differentiation both in wild-type and in p21WAF1/CIP1 null osteoblasts [33].

Osteoblastic differentiation with sample 1 at the end of 24 hrs was 0.0837 over all %cell inhibition was 75.67, with sample 2 at the end of 24 hrs it was 0.2402 % cell inhibition over all was 57.67% for sample 3 at the end of 24 hrs 0.1643 overall was 75.62%, change in concentration could not create any favourable condition for the proliferation of the cells, secondly the other condition was the use of cancerous cell line, turmeric previously showed to have tremendous effect on killing cancer cells [25,26], which also might have contributed for the negative outcome of the results, studies have to be undertaken on a different cell line which has normal osteoblasts, individual studies of these extracts have revealed that turmeric showed cytotoxicity in lower and higher concentrations but aloe vera extract acemannan showed cell proliferation at lower concentrations cytotoxity occurred at higher concentrations (Table 2 and 3).

Plant Name	Concentration (µg/ml)	Absorbance 590nm	% Inhibition	IC_{50}
Control	0.0	0.495	0.00	36.13 µg/ml
Curcumin	1.5	0.482	2.70	
	3.1	0.449	9.37	
	6.3	0.403	18.65	
	12.5	0.346	30.16	
	25.0	0.279	43.68	
	50.0	0.164	66.90	

Table 2: *Individual analysis of acemannan and curcuminoids.*

Plant Name	Concentration (µg/ml)	Absorbance 590 nm	% Inhibition	IC_{50}
Aloe vera	0.0	0.347	0.00	NA
	1.5	0.341	1.64	
	3.1	0.334	3.66	
	6.3	0.329	5.11	
	12.5	0.312	10.01	
	25.0	0.295	14.91	
	50.0	0.243	29.91	
	100.0	0.207	40.29	

Table 3: *Individual analysis of acemannan and curcuminoids.*

Conclusion

The present study can be concluded that, the effects of Hydrophilic gel and its contents on osteoblast cells seeded directly onto Titanium discs. In respect to cell morphology, cell density and the effect on the surrounding pH, the Hydrophilic gel formulated showed no cell proliferation. However, the mechanism of cell stress induction and cytotoxicity needs to be further studied and other extracts also should be considered to enable prediction of possible health risks, and to better evaluate the methods to enhance osseointegration.

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