Prospects of Extremophiles & Sulfated Polysaccharides in Bionanotechnology & Biomedicine

Dr. Sreejith Raveendran, Ph.D.
Post Doctoral Researcher, Bionanoscience
Bio-Nano Electronics Research Center
Graduate School of Interdisciplinary New Science
Toyo University, Japan
Sulfated Polysaccharides (SPS) are complex PS molecules with excellent physico-chemical properties & bioactivities. On the basis of origin they are classified as Plant, Animal, Microbial & Chemically synthesized.

SPS are good matrix materials for various nanoformulations: nanoparticles, micro-emulsions, liposome stabilization, polymeric micelles, hydrogels, polymeric scaffolds, functionalization molecule, etc..

PS are widely used in the drug delivery appltn. as polyelectrolytes forming multilayers based on various factors like hydration, internal composition, charge distribution, chemical modification, etc..

They posses glycosidic linkages that can be cleaved easily by hydrolase enzymes, hence they are biodegradable.

They are highly polyanionic due to the presence of the negatively charged functional gps like $\text{SO}_4^{2-}$, $\text{COO}^-$, $\text{PO}_4^{2-}$, etc. hence they are widely used for polyelectrolyte complexation processes.

Many SPSs are well studied for their enumerable biological properties and therapeutic importance. Naturally extracted SPSs posses anticancer, antiangiogenic, antiviral, anti-inflammatory, antithrombogenic, antiparasitic, antioxidant, antihelminthic, antihemolytic properties, etc...

Both, molecular weight and the degree of sulfation plays vital roles in deciding the bioactivity of the specific SPS. They can be altered according to the need by chemical sulfation.
Few Commercially Important Extremophiles...

- Used in biotechnology, medicine, food & cosmetics.
- Include vitamins, amino acids, peptides, proteins, essential fatty acids (DHA, ARA, other PUFAs), Polysaccharides, pigments (β-carotene, astaxanthin), Phicobiliproteins etc..
- Certain microbial biomass are also used for food and industrial applications. eg: *Spirulina*
- Generally 7 types of extremophiles:
  - Thermophiles- Temp. above 60°C
  - Psychrophiles- Temp. below 0°C
  - Halophiles- High Salt conditions
  - Alkaliphiles- pH above 10- alkaline pH
  - Acidophiles- pH close to 0- acidic pH
  - Metallophiles- Requires heavy metal conc.
  - Barophiles- High hydrostatic pressure about 1000 atm;
- Others like Xerophiles( water scarce conditions), Anoxiphiles (oxygen deprived conditions) etc.
**Microbiology - Bacterial Culture & EPS Production**

- *Halomonas maura* & *Halomonas eurihalina* were grown in MY medium; EPS were extracted by cold ethanol extraction; Purified by dialysis & lyophilized.

- Structure of bacteria was analyzed using TEM & SEM studies.

- TEM - Negative staining; Ultra thin sectioning & 3D electron tomography; SEM - by glutaraldehyde fixation.

- EPS characterization - XPS, FTIR - Spectra was recorded.

- Mauran (MR), consists of nanosized globules joined together by a network of sticky fibrils; whereas EPS *H. eurihalina* (HeEPS), were found as uniform outer layer that surround the entire bacterium in a sticky nature.

- On characterization of the EPS, it was found that MR contains C, O, N, S, & P; high degree of sulfation; COO\(^{-}\) and SO\(_4^{2-}\) were found as major functional gps; FTIR analysis revealed the typical polysaccharide nature of the MR with sulfate functionalization, to demonstrate SPS.
Electron Micrographs of *Halomonas*

Figure 2. SEM images of *Halomonas maura* showing mauran accumulation (A, B); TEM images of ultra thin section of *H. maura* showing mauran (C, D); TEM images of negatively stained *H. maura* showing mauran (E, F).

Figure 3. 3D TEM images of *Halomonas maura* showing MR accumulation: G, TEM image of ultra thin section of *H. maura* showing MR; H & I, 3D images of Image G.

Figure 3. SEM images of *Halomonas eurihalina* showing EPS accumulation (A, B); TEM images of ultra thin section of *H. eurihalina* showing EPS (C, D); TEM images of negatively stained *H. eurihalina* showing EPS (E, F).

Figure 4. XPS Spectra for EPS from *H. maura* & *H. eurihalina*.

Figure 5. FTIR spectra for EPS from *H. maura* & *H. eurihalina*.
Mauran Based Nanoparticles for Sustained Drug Delivery, Cancer Chemotherapy & Bioimaging

Synthesis of MRCH nanoparticles

- Extraction of MR from H. maura; Subjected to polyelectrolyte complexation with Chitosan (CH) in 1% acetic acid.

- NH$_2$ gp of CH and COOH gp of MR undergo ionic gellation to form MRCH. Stable quasi spherical nanoparticle clusters were formed during ionic gellation; Size was of 30-200 nm; positive zeta potential of 27.5±5 mV.

- Characterization of MRCH nps: SEM, TEM- EDS, XPS, FTIR, UV-Vis.

- Encapsulation of test drug 5FU; Sustained drug delivery, release kinetics and concentration of release were found out using RP-HPLC.

- Biocompatibility, Cytotoxicity & Anticancer activity of 5FU -MRCH nps were analyzed using in vitro cell culture studies; mouse fibroblast cells, L929; breast adenocarcinoma MCF7; & gliosarcoma, G1 cells were used; 5 consecutive days study was performed for MCF7 cells.

- Fluorescent dye tagging; Fluorescein isothiocyanate (FITC) labelled CH; Sypro Ruby(SR) labelled MR were separately used; green & red fluorescing MRCH nps were synthesized.

- Bioimaging using fluorescent nps; Nontoxic live cell bioimaging were performed using confocal microscopy.

- Effect of pH was checked for stable nps formation; acidic- neutral pH were checked and found that acidic pH is appropriate for MRCH nanoparticle formation. (Table. 1)

- Effect of concentration of the solutes were also checked by keeping one the solute conc. stable; appropriate ratio was found to be 1:1. (Table. 2)

- FTIR spectra shows the successful ionic gelation & nanoparticle formation during polyelectrolyte complexation between MR & CH.
Characterization of MRCH nps

Interaction of COOH and NH$_2$ functional gps were confirmed using SEM-EDS.

The presence of SO$_4^{2-}$ gp in the surface as well as the density of NH$_2$ & COOH gps were found by the S, N & C intensity observed.

XPS spectra shows the successful encapsulation of 5FU as the test drug with in the MRCH nps by the presence of F at 23 eV for F 2s.
• UV-vis spectra of MR, CH, MRCH, 5FU, 5FU-MRCH, MRCH-FITC & FITC were taken.

• Drug release kinetics revealed the sustained release of 5FU from MRCH nps within 10-12 days of incubation under 3 different conditions.

• 2 sets of samples were employed at 37°C under 100 rpm uniform shaking
  • New set of 5FU-MRCH nps
  • Three months Old 5FU-MRCH nps.

• Three different pHs - 4.5, 6.9 & 7.4

• Different from other conventional systems burst release was observed only after 3rd day in old sample at pH4.5 and 5th day for all others.

• ~60% of the drug was released under acidic pH of 4.5, which favors the sustained release of drug within 10 days of incubation.

• Thus, favoring sustained and controlled release profile for cancer chemotherapy.
### In vitro Cytotoxicity Assay

- Highest conc. of the MRCH nps on L929 cells showed 85% of viability, showing the biocompatible & non-cytotoxic nature of MRCH void nps.

- In case of 5FU- MRCH nps, the 5 day studies revealed the better sustained killing of MCF 7 cells rather than a sudden killing as observed in free 5FU.

- Ultimately, in both cases the cell viability could be reduced to an extend of ~11%. Thus showing the positive effects of sustained & controlled killing.

- The harmful side effects of free 5FU can be greatly minimized on nanoencapsulation within MRCH nps.

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**Figure 7:** Results of cytotoxicity assay: A. MR/CH nanoparticles on L929 cells; B. 5 days study of free SFU on MCF7 cells; C. 5 days study of 5FU loaded MR/CH Nps on MCF7 cells.
Bioimaging & Flow Cytometry using Fluorescent MRCH nps

- FITC labelled CH was used in Polyelectrolyte complexation.
- MRCH-FITC nps were formed and UV-vis spectra was taken.
- Treated with MCF 7 cells for studying cellular binding and uptake.
- Few cells were absorbed by MCF7 cells and most of them were adsorbed to the cell surface within 24h of incubation.
- Epifluorescent microscopy showed the successful binding & uptake of MRCH- FITC nps.
- Cell were found viable even after the uptake of void MRCH-FITC nps for 48 hrs. Thus ideal for live cellular imaging.
- Flow cytometry data showed the differences in fluorescence intensity, due occurrence of various events.
- Several events (H1-H4) were observed due to the difference in absorption of fluorescent nps in the culture.
- SR-MRCH & MRCH-FITC nps were analyzed using Flow cytometry.
Figure 9: Flow cytometry data of MR/CH- FITC nanoparticles and SR- MR/CH nanoparticles treated- A & B, L929; C, MCF7

Figure 9: Flow cytometry data of MR/CH- FITC nanoparticles and SR- MR/CH nanoparticles treated- D, MCF7; E & F, G1 cells
• SR-MRCH nps & MRCH-FITC nps were treated with L929, MCF 7 & G1 cells for 24h.

• L929 cells showed more uptake than MCF7 & G1 in 24h

• SR labelled cells were fluorescing in Orange red at 561 nm.

• FITC labelled cells were fluorescing in Green at 493 nm.

• DAPI stained cells showed that the absorption of MRCH nps does not cause any cytotoxicity and keeps the cells viable.

• Results obtained via confocal imaging supports the 5 day cytotoxicity study that only few particles will be absorbed by MCF7 & G1 in 24h unlike L929 cells.
In vitro Evaluation of Antioxidant, Antithrombogenic, Antiangiogenic Properties & Hemocompatibility of Extremophilic Polysaccharides

Antioxidant, Antithrombogenic, Antiangiogenic properties & Hemocompatibility of MR polysaccharide

• PS are generally good bioactive molecules with enumerable biological properties: Antioxidant, anticancer, antiviral, antiparasitic, antihelminthic, antithrombogenic, antiangiogenic, etc..

• Both MR & MRCH nanoparticles were separately analyzed for Antioxidant behavior.

• Also, MR was evaluated for antihemolytic, antithrombogenic and antiangiogenic effects.

• 5 different parameters analyzed:
  • Lipid Peroxidase (LPO) Assay
  • Reduced Glutathione (GSH) Assay
  • Glutathione Reductase (GR) Assay
  • Glutathione Peroxidase (GPx) Assay
  • Superoxide Dismutase (SOD) Assay

• All these enzymes are direct monitors of the antioxidant activities that takes place in the living tissues.

• Absorption studies were performed using fluorescent -labeled MR & confocal microscopy.
LPO for MR

- Cell membrane damage due to oxidation of membrane lipids causes free radical electron removal from cell membrane resulting in lipid peroxidation.
- LPO assay characterized by oxidation of Thiobarbituric acid (TBA) to form a fluorescent product Melondialdehyde (MDA) & measured @ 532 nm.
- Results suggest that the MDA concentration was well correlating with ctrl value except in the cell conc. 1 mg/ml. Thus confirms the antioxidant behavior of MR.

GSH for MR

- Moron *et al* method used to assay GSH level; dithio-bis-nitrobenzoic acid (DTNB) reacts with GSH to get a spectrophotometrically detectable pdt.
- Change in absorbance is a linear function of the GSH concentration, expressed in nmol/mg of protein.
- It is evident that the Conc. of MR is not affecting the action of GSH, since the test values are far above the control value in tissue; whereas the cells show a tendency to acquire the control limit only when the conc. increases, this variation may be due to decreased protein content.

GR for MR

- GR catalyses the rxn of converting oxidized glutathione (GSSG) to reduced glutathione (GSH).
- Antioxidant activity of MR controls the conversion of NADPH to oxidized form their by maintaining the GSH level.
- In both cell & tissue homogenates the GR level is below the control value, except for the highest conc. in cells.
- The Assay measures the amount of autooxidation of pyrogallol by SOD and the rxn pdt is read @ 420 nm.

GPx for MR

- GPx catalyses the rxn of converting H$_2$O$_2$ into water and lipid peroxides to corresponding alcohol.
- MR is not interfering with the action of GPx since the test values are below the ctrl in tissue, whereas in cells the value tend to rise after 100 µg/ml conc.

SOD for MR

- Results indicates that there is a slight level of increase in SOD in cells, whereas as it is comparable in tissue; but an insignificant increase was observed at 500 µg/ml.
Blood compatibility of the MR can be evaluated by its hemolytic index.

Antithrombogenic effect can be found out using the whole blood clotting time; enhance clotting time has been observed for MR conc. above 0.25 mg/ml.

Hemocompatibility & Whole Blood Clotting Time

Table 1: Whole blood clotting time shown by various concentrations of MR in comparison with control.

<table>
<thead>
<tr>
<th>MR concentration</th>
<th>Control</th>
<th>2mg/ml</th>
<th>1mg/ml</th>
<th>0.5mg/ml</th>
<th>0.25mg/ml</th>
<th>0.1mg/ml</th>
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</thead>
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<tr>
<td>Seconds</td>
<td>335</td>
<td>&gt;480</td>
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<td>450</td>
<td>420</td>
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<td>&gt;480</td>
<td>&gt;480</td>
<td>&gt;480</td>
<td>480</td>
<td>410</td>
</tr>
</tbody>
</table>

1mg shows hemolytic index of 1.059±0.263%; Std = 5%

MR Absorption Studies

SR-MR, Orange- red fluorescent MR particles were treated with L929 cells 24h. Stained with DAPI & Mitotracker green. Confocal images were recorded.

Mr absorption images supports the Antioxidant and Cytotoxic studies that on MR treatment, cell membranes are not damaged rather they maintain the integrity of the cell. Hence proved non-cytotoxic and biocompatible.
Antioxidant defense mechanism of MR/CH nanoparticles

Cells showed slight difference that is comparable, but it is well below the ctrl; in case of tissue the test values were well below the ctrl. MRCH nps boosts the antioxidant property by restricting the free radical generation.

Although the ctrl value is higher than the test values in tissue, the increase in conc. of GSH shows a minimal oxdtn. & protective effect. Where as the cell value from 500µg slight showed an increase.

An insignificant increase was observed in case of cells; whereas the tissue values showed a decreased profile of GR conc. in comparison with its ctrl.

SOD production was well below the ctrl value in case of cell homogenate; where as in the tissue homogenate it was found that the highest conc. was capable of raising the SOD value to an insignificant level.

Thus, concluding that the MRCH nps can be safe below the concentration of 500µg under in vitro conditions.
**Antiangiogenic Study for MR & HeEPS**

- Development of blood vessels or vascularization plays an important role in cancer related metastases.

- Angiogenesis is an inevitable factor for the spread of cancer or solid tumors; helps in $O_2$ & nutrient supply; SPS inhibits the tumoral angiogenesis.

- MR & HeEPS showed successful inhibition of HUVEC cell angiogenesis under *in vitro* conditions, in preliminary studies.
Mauran Based Biocompatible Nanofiber Scaffolds For Tissue Engineering Applications

Biocompatible MR/PVA Nanofibers for Tissue Engineering Applications

- Bacterial PS based nanofibers were introduced for the first time into science & technology.
- Thin uniform nanofibers were spun using homogenous MR & PVA solution using electrospinning.
- Solubility tests were performed for MR in various organic and inorganic solvents.
- Rheology was analyzed; SEM, XPS, FTIR characterization were done.
- Biocompatibility assay was performed using L929 & mesenchymal stem cells, KUSA.
- Cell adhesion, proliferation and differentiation studies were performed.
- Can be applied for biomedical and tissue engineering applications.

FTIR characterization reveals the presence of S & N from MR on MR/PVA nanofibers.

Rheology of the spinning solutions were tested; MR at various concs. were employed in the order: 6mg, 15 mg, 20 mg & 30 mg of MR containing nanofiber preparing soltn.

It was observed that the increasing conc. of MR greatly alters the rheology of the spinning solution; this helped in the uniformity of the nanofibers synthesized.
• SEM images shows identical morphological features for all NFs with different concs. with large aspect ratio.

• 6 mg conc. NFs were having uniform diameter of 110 nm. On increasing the conc. from 6 to 30 mg, the morphology showed similar smooth & uniform diameter with interconnected pores.

• MR/PVA NFs were much broader than the pure PVA NFs, which were having certain sticky appearance in the fiber junctions.
Biocompatibility Assay

- Biocompatibility studies were performed using alamar blue assay.
- Three days study were performed using mouse fibroblast cells & mesenchymal stem cells.
- Studies showed that the increasing conc. of MR in the MR/PVA fibers had a great effect on the cellular growth.
- Most of the conc. registered over growth when compared with control wells. Thus proves that MR/PVA NFs are excellent for enhanced cellular growth.
- Both the cell lines, L929 & KUSA were showing similar results; Highly biocompatible & non-cytotoxic in nature.

Adhesion, Proliferation & Differentiation Studies
Bioreduction and Passivation of Gold Nanoparticles Using Mauran & Photothermal Ablation of Cancer cells

“Bioreduction and stabilization of gold nanoparticles using extremophilic exopolysaccharides and photothermal ablation of cancer cells”. Sreejith et al, (Under communication).
In this study, we found an alternative to chemical reduction of AuNps to bioreduction using bacterial SPS for the 1st time.

- We correlate the reducing efficiency, cytocompatibility and photo ablation property of MRAuNps with conventionally produced AuNps.

- MR was used in 3 different concs.; NaBH₄ + 5 different concs. of MR were separately prepared.

- In former case, MR was acting as the sole reducing agent and in later case MR was acting as a stabilizing agent.

- In both cases the Nps formed where of different morphologies. Characterized using UV-vis, TEM, EDS, XPS, Zetasizer.

- Biocompatibility was assessed using L929 cells; Anticancer therapy by photo ablation was done with GI & MCF 7 cancer cells.

- Photablation studies showed increase in temp with in seconds, which was sufficient to kill the cancer cells in the in vitro conditions.

- Metal binding property of MR has been exploited here; Polyanionic nature of MR plays a vital role in the reduction and passivation of the AuNps.

- MR solution in the presence of HAuCl₄ on heating at 80°C can undergo detrimental effect, this may result in the formation of constituent sugars and thereby the redtn of the Au soltn.

- Red shift was observed in the case of MR reduced AuNps from the NaBH₄ reduced AuNps. Peak was broadened on Mr Passivation.
Figure 3: TEM images of MRAu Nanoparticles synthesized using MR as stabilizing agent & NaBH₄ as reducing agent, forming various spacial arrangements and structures.

Figure 4: TEM images of MRAu Nanoparticles synthesized using mauan as reducing agent, forming various spacial arrangements and structures.
Biocompatibility study

- Bare AuNps where found cytotoxic and on passivation the toxicity was drastically reduced.

- Highest conc. showed ~ 70% of viability.
100µg/ml conc. of AuNps showed 53.1 ± 5°C in 10 min of laser irradiation.
• MCF 7 & GI cells were killed due hyperthermia generated during the photothermal ablation.

• Many factors affect the efficiency of killing: Laser type, location of AuNps, Conc. of Nps, state of AuNps, i.e. aggregates or dispersed etc..

• In conclusion the bacterially generated PS are well suitable for bioreduction and passivation for AuNps than any toxic chemicals or molecules with cytotoxic effects.

• Moreover, the MRAuNps are competent with the conventionally produce AuNps in generating hyperthermia with an added advantage of biocompatibility.
Enhancing biocompatibility of Quantum dots by bacterial polysaccharide conjugation for 
in vitro bioimaging

“Enhanced biocompatibility of quantum dots by extremophilic polysaccharide for nontoxic bioimaging”. Sreejith et al. (Under communication).
Enhanced Biocompatibility of QDs on MR conjugation

- Metal nano crystals, Quantum Dots (QDs) are excellent fluorescent biomarkers for imaging and clinical diagnostics.
- Toxicity is the most perturbing issue for *in vivo* applications; stabilization with a biologically active stable PS enhances the biocompatibility.
- MR conjugation has increased the cytocompatibility with same fluorescing ability for bioimaging.
- Heavy metal binding property of MR was exploited for the current application.
- ZnS: Mn QDs were synthesized & treated with MR solution.
- Characterized using UV-Vis, Fluorescence Spectroscopy, FTIR, TEM & EDS.
- Biocompatibility was evaluated using L929 cells.
- Imaging was done using L929 & MCF7 cells.

**UV-vis & PL**

- 5 nm sized bare ZnS: Mn QDs on MR stabilization yields 10-20 nm MR-QDs.
- 2mg/ml conc. of MR was used for 0.5 mg of QD.
- 2 sets of samples were used: freshly prepared MRQD & 3 months stored MRQD to show photo stability on storage.
- A shift in absorption peak from 320nm to 325 nm shows the successful coating of MR.
- An Orange-red emission was observed at 580nm in PL spectra.
Figure 4: TEM micrographs showing:
A, ZnS: Mn QDs;
B & C, Clusters of MR-QDs;
D, individual MR-QD particles.

Figure 6: FTIR spectra for MRQD (1), QD (2) & MR (3)

Figure 5: A, TEM-EDS spectrum showing the constitutive elements detected in MR-QD nanocrystals; B & C, SEM micrographs showing clusters of MR-QDs under different magnifications.
Bare QD nano crystals were highly cytotoxic and it could show only ~44% cell viability in the least test con. of 0.05 mg/ml.

On MR stabilization the compatibility increased and the % of viability was ~80% under highest test conc. of 1mg/ml

Fluorescence intensity was almost equal in both cases when flow cytometry spectra was analyzed. 10^3-10^5 cells were found fluorescing.

This shows that the 3/4 of the cells treated (10^7 cells) were found fluorescent.

Bright orange-red fluorescence were observed under epifluorescent & phase microscope. DAPI stained cells revealed that after 24h of MRQD treatment, cell were remaining alive.

Thus, confirming the enhancement of biocompatibility and reduction of cytotoxicity by passivation of MR as a biocompatible bacterial PS.

**In vitro Cell Studies**

**Figure. 7:**
*In vitro* cytotoxicity of QD, MRQD and MR using L929 mouse fibroblast cells

**Figure. 8:** Flow cytometry spectra showing the uptake of fluorescent MRQD particles by L929 cells. A, MRQD.New set; B, MRQD.Old set.

**Figure. 9:** Phase contrast & epifluorescent imaging of MRQD treated L929 cells & MCF7 cells. A & D, Bright field image of L929; B & E, Phase contrast image of L929; C & F, Epifluorescent image of L929; G & I, Bright field image of MCF7; H, Phase contrast image of MCF7; J, Epifluorescent image of MCF7;
Ecofriendly Route for Synthesis of Highly Conductive Graphene using Extremophiles for Green Electronics and Bioscience

Ecofriendly Reduction of GO by Extremophiles

- Highly conductive, biocompatible, with large surface area graphene can be produced by reduction of GO using extremophilic bacteria.

- *H. maura* & *H. eurihalina* were used under aerobic & anaerobic conditions for GO reduction.

- Degree of reduction was analyzed & the characterization was performed using TEM, SEM, AFM, UV-vis, XPS, & Raman.

- Electrical measurements were performed using 3-probe method; Field effect transistors (FET) were made; increase in conductivity from GO to graphene was recorded.

- Biocompatibility of the graphene synthesized using bacterial reduction was found out using *in vitro* cytotoxicity assay with mouse fibroblast cells.

- A cost effective, ecofriendly, nontoxic, bulk reduction of GO to produce large area graphene was introduced for green electronics.
Figure 1: (A), Extremophilic reduction of ten times diluted concentration of GO- A1, H. eurihalina reduced GO; A2, H. maura reduced GO & A3, GO control; (B), UV-Visible spectrum- B1, GO spectrum; B2, ERGO spectrum; B3, MRGO spectrum; (C), XPS analysis spectrum for C 1s- After anaerobic GO reduction.

Figure S1: GO reduction at 1 mg/ml concentration- (a) Image taken immediately after bacterial inoculation (b) Image taken after 10 days of incubation. (A- GO; Control; B & C- ERGO; D & E- MRGO).

Figure S2: XPS analysis spectrum for C 1s- after aerobic GO reduction.

Figure S3: TEM micrographs of RGO (a), MRGO; (b), ERGO.
**TEM-SAED-AFM-RAMAN**

- TEM-SAED pattern shows the unit crystalline pattern of graphene, depicting the single- double layers.
- Ratio of the intensity of the Raman peaks were compared to evaluate the rate of reduction.
- AFM results supports the TEM results in no: of layers.
- 1.7 nm & 2.7 nm were the thickness of the graphene sheet.

**Table 1.** The positions of G and D bands in GOctrl, ERGO and MRGO. The intensity ratios of D band and G band are also given.
Electrical Measurements

- The contact resistance of the samples were negligible to discuss the electrical properties.

- The resistivity of GO sheet was $3 \times 10^3 \Omega \cdot m$; after redtn it decreased to $1.3 \times 10^{-2} \Omega \cdot m$ & $2.5 \times 10^{-2} \Omega \cdot m$ for MRGO & ERGO respectively.

- An increase of $10^4$- $10^5$ fold in conductivity of RGO from GO attributes to the successful redtn of GO by bacteria.

- Biocompatibility graph shows that the cell viability increases as the conc. of ERGO & MRGO is increasing.

Thus, we introduce the extremophilic mode GO reduction for producing biocompatible graphene for green electronics.
Conclusion

• Remarks successful outcomes of the bionano fusion based on extremophilic bacteriology & nanotechnology.


• Ultimately, we have achieved several modes of green nanotechnology techniques, which utilizes biocompatible, easily biodegradable, biomaterials.

• Application of biologically active Ext. bacterial SPS in biopharmaceutical and nano industries made feasible. Bulk production; easy culturing; less culture time; greater yield; less cytotoxicity; versatile physico-chemical properties; enumerable bioactivities are the highlights of Ext. Bacterial SPS over other types of SPS.

• Similarly application of extremophiles in GO redtn can be a breakthrough for generating user friendly, nontoxic graphene for nanoelectronics.

• Extremophiles as the first living organisms & as the ultimate living organisms, can revolutionize the world of material science & technology as an indepletable source of energy forever.

• It offers a hand full of discoveries that may definitely change the trends in bionanotechnology research in near future.
1. Bacterial polysaccharide based nanoparticles for sustained drug delivery, cancer chemotherapy and bioimaging.

2. Biocompatible nanofibers based on extremophilic bacterial polysaccharides from Halomonas maura.
Sreejith Raveendran, Brahatheeswaran Dhandayuthapani, Yutaka Nagaoka, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. *Carbohydrate Polymers*, 2013, 92; 1225-1233. (Research Article)

3. Pharmaceutically versatile sulfated polysaccharide based bionano platforms.

4. Ecofriendly route for synthesis of highly conductive graphene using extremophiles for green electronics and bioscience.

5. Effect of mauran nanoparticles on oxidative stress and critical evaluation of its differential therapeutic index on anticancer mechanism using glioblastoma and breast carcinoma cells.
Sreejith Raveendran, Vivekanandan, Yutaka Nagaoka, Takahiro Fukuda, Seiki Iwai, Yasushi Sakamoto, P. V. Mohanan, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (Communicated). (Research Article)

6. *In vitro* evaluation of antioxidant defense mechanism and hemocompatibility of mauran.

Sreejith Raveendran, Sivakumar Balasubramanian, Aswathy Ravindran Girija, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (Communicated). (Research Article).
8. Bioreduction and stabilization of gold nanoparticles using extremophilic exopolysaccharide and photothermal ablation of cancer cells.

Sreejith Raveendran, Neha Chauhan, Vivekanandan Palaninathan, Yutaka Nagaoka, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (To be communicated). (Research Article)


Sreejith Raveendran, Srivani Veeranarayanan, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (To be communicated). (Research Article)

10. PEG Coated Biocompatible Cadmium Chalcogenide Quantum Dots for Targeted Imaging of Cancer Cells.

11. Multifunctional biocompatible fluorescent carboxymethyl cellulose nanoparticles.

12. Aptamer conjugated paclitaxel and magnetic fluid loaded fluorescently tagged PLGA nanoparticles for targeted cancer therapy.

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