Interfacial properties as predictors of radioresistance in cervical cancer

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Abstract

The prediction of radioresistance of tumours, early in the course of radiotherapy, may help clinicians in deciding the optimal treatment strategy for each case. This study was carried out to investigate an in vitro technique to predict radiosensitivity, after a single radiation dose of 2 Gy in cervical cancer. Langmuir films of tissue homogenates of biopsy samples from 20 cervical cancer patients treated with radiotherapy alone and 15 normal controls were evaluated. The tensiometric profiles before and after giving 2 Gy of radiation, were compared with that of controls and were correlated with the clinical radioresponsiveness evaluated on completion of the radiotherapy course of 70–78 Gy over a period of 50–55 days. The tensiometric profiles measured after a single dose of radiation can be used to fingerprint the clinical radioresponsiveness of the cervical cancer tissues. The hysteresis of the monolayers of completely radioresponsive post-radiotherapy tissue homogenates was 5.8 times greater than that of partially radioresponsive post-radiotherapy tissue homogenates and was statistically significant using Mann–Whitney test (p < 0.05). From our results, the following tensiometric criteria for prediction of radioresistance emerge. After first dose of radiation, if the minimum surface tension of tissue homogenate is greater than 50 mN/m and hysteresis area is less than 20 µJ those tissues will be in the partially radioresponsive and for completely radioresponsive tissue homogenates, the minimum surface tension will be less than 47 mN/m and the hysteresis area will be greater than 33 µJ. The cholesterol and phospholipid content of radioresponsive cervical cancerous tissues after radiotherapy was found to be 1.2 and 2.2 times lower than that of the untreated tissues and due to lower lipid content organic phase surface activity of radioresponsive cancerous tissues after radiotherapy was less than that of the untreated tissue organic phase. The radiation induced tensiometric profile changes of radioresponsive cervical cancerous tissues can be correlated to the radiation induced lipid profile changes. This technique, due to its simplicity and high precision, can serve as a predictive tool for radioresponsiveness and is easily translatable to the clinical setting. Randomized large sample trials are necessary to validate this technique further and help in the translation from bench to clinics.

Keywords: Tissue monolayer; Surface pressure–area isotherm; Hysteresis; Cervical cancer; Minimum surface tension; Radioresponsiveness

1. Introduction

Cancer of uterine cervix remains a major health problem throughout the world, accounting for almost 6% of all malignancies in women. In incidence and in mortality it is second only to breast cancer and it has a high prevalence especially in Asia [1,2]. In case of cervical carcinoma, radiotherapy is a curative option for the early stages of cancer and radioresistant tumours limit local control and survival [3]. The time taken for recognizing treatment failure may provide further opportunity for metastatic spread. Therefore, it is desirable for oncologists to predict the radiosensitivity of the tumours early in the course of radiotherapy, which may allow them to individualize and modify the treatment strategy [4,5]. In cervical cancer, response after radiotherapy is often assessed subjectively by pelvic examination [6]. Clonogenic survival assay is the most common method to measure in vitro radiosensitivity but is time and resource consuming [7]. However objective measures are needed for radiosensitivity prediction and efforts to develop a rapid and complete marker of radiosensitivity are on.

The lipid and protein profiles are altered during carcinogenesis and these biochemical changes may be suggestive of cancer progression and development. If a single technique can monitor these complex biochemical changes as a whole, then it would be preferred over the need for monitoring several biomarkers indi-
vidually. Lipids are amphiphilic and surface active; so changes in lipid composition can be monitored by studying the interfacial properties of cancerous and normal tissues. Monolayers at an air–liquid interface are sensitive models for understanding the interfacial behavior of biological systems and can be used to detect even subtle changes in lipid profiles. Tissue tensiometry using such monolayers has been established as a technique to differentiate between tissue types as well as between diseased and normal tissues [8]. Characteristic tensiometric profiles have been identified for cancerous and normal human cervical tissues [9]. In this paper, we evaluated tensiometric profiles, using a monolayer study, as predictors of radiosensitivity in human cervical cancer. We characterized the pre- and post-radiotherapy human cervical tissues from same patients by the Langmuir film technique and compared the results with that of normal human cervical tissue monolayers. The tissues were classified based on their tensiometric profiles and compared with the actual clinical radiosensitivity of the tissues evaluated at the end of the entire course of radiotherapy of a total dose of 70–78 Gy. Efforts have been made to finger print the radioresistant and radiosensitive cancerous cervical tissues based on several tensiometric parameters using a small sample pilot study for establishing proof of principle.

2. Materials and methods

2.1. Collection of tissues

Human biopsy specimens of cervical cancerous tissues (n = 20) and normal cervical tissues (n = 15) were obtained from the Radiation Oncology Division of Nanavati Hospital, Mumbai, India. The use of human tissue biopsies was approved by the ethical committee of the hospital in accordance with the ethical standards as formulated in the Helsinki declaration. All cancer samples were of stage III squamous cell carcinoma of cervix and collected from patients, who received only radiotherapy, in the age group of 30–80 years. Samples were collected prior to the start of radiotherapy and after giving a radiation of 2 Gy (first dose) to obtain the pre-radiotherapy and post-radiotherapy samples, respectively. The details of the radiotherapy are described in the next section. The normal control samples were collected from hysterecomy patients having non-cervical disorders. All the normal samples were reported to be free from malignancy by histopathology analysis and the cervical portion was normal or mildly inflamed.

2.2. Radiation treatment

All patients were irradiated in Theratron 780C (Atomic Energy of Canada Ltd., Chalk River, ON, Canada), a telecobalt equipment. Patients were treated at a dose rate of 1.2–1.4 Gy/min with a parallel opposed field, box technique or with multiple beams to encompass the entire pelvic disease. The upper margin of the field was placed at the L5/S1 junction, whereas the lower margin included the lowest palpable disease with a centimeter of margin. All patients received 50 Gy of radiation in five weeks, with external beam radiotherapy. It was followed by intracavitary radiation after a gap of seven to ten days. A prescribed dose of 20 Gy was delivered to a reference point A with a low-dose-rate radiation. Some of the patients received 28 Gy in two insertions to the point A. The overall treatment duration was 50–55 days with a total dose ranging from 70–78 Gy. The post-radiotherapy samples were collected after 24 h of administration of the first dose of radiation (2 Gy). The clinical response assessed at the end of radiotherapy after the entire radiation dose was categorized as complete or partial by the WHO criterion [10]. The physician evaluating the clinical responsiveness was blinded to the tensiometric profiles of the patients.

2.3. Preparation of tissue samples

After collection, all the tissue samples were washed thoroughly with normal saline, dried on a tissue paper and weighed. The weighed samples were processed by using liquid nitrogen and dissolved in measured volumes of normal saline to form a tissue homogenate of known concentration. The tissue homogenate was kept at −10°C till experimentation and all the experiments were performed within 10 days of sample collection. It was confirmed that the surface activity of fresh and stored tissue homogenate was not altered within 10 days. The cellularity of the tissues was evaluated and the cell counts in equal weights of cancerous and normal cervical tissues were statistically similar. Thus our tensiometric measurements were standardized in terms of cell counts as well as weight of tissue. All samples and their radioresponsiveness were blinded for tensiometric evaluation.

2.4. Extraction of tissue homogenates

The organic phases containing the lipophilic components and the aqueous phases containing the lipophobic parts of the tissue homogenates were separated by using Bligh–Dyer extraction procedure. Briefly, 0.8 ml of the tissue homogenate was treated with chloroform and methanol in the ratio 1:2 and agitated well to get a single phase. To this 1:1 chloroform:water was added and agitated well for 5 min followed by centrifugation at 100g for 10 min, to achieve good phase separation. The separated organic and aqueous phases were stored at −10°C till experimentation. All measurements were conducted within 24 h of extraction.

2.5. Laboratory chemicals

AR grade methanol and acetone for cleaning of Langmuir trough and AR grade sodium chloride for normal saline preparation and potassium dihydrogen phosphate for phosphorus assay standard were purchased from SRL, Mumbai, India. HPLC grade dichloromethane, ortho-phthalaldehyde, glacial acetic acid, concentrated sulphuric acid, malachite green, concentrated hydrochloric acid, polyvinyl alcohol, perchloric acid, hexane, potassium hydroxide and ammonium molybdate (for lipid quantification) were purchased from Loba Chime, Mumbai, India. Cholesterol for standard curve in cholesterol assay
was obtained from Sigma–Aldrich Co. (St. Louis, USA). High purity water purified by a Milli Q Plus water purifier system (Milli Pore, USA), with a resistivity of 18.2 MΩ cm, was used in all experiments.

2.6. Monolayer experiments

Monolayer studies were performed by using a computer controlled LB film balance (Mini Trough, KSV Instruments, Finland). The trough is placed on an antivibration table, which is enclosed by an environmental chamber. The Teflon coated trough is equipped with two delrin barriers (for monolayer compression and expansion) and the entire trough is surrounded by a water jacket, providing temperature control. A Wilhelmy plate balance with a platinum plate (19.62 × 10 mm) is used for sensing the surface pressure.

Before each monolayer experiment, the trough and barriers were thoroughly cleaned by organic solvents (methanol and acetone) and deionized water in sequence several times. Highly pure deionized water, having resistivity 18.2 MΩ cm, was the subphase in all experiments. The temperature of the subphase was maintained at 37 ± 1°C with the help of an external circulating water bath. The surface was cleaned with the help of an aspirator and the cleanliness was ensured by a zero reading of the surface pressure. One mg of tissue homogenate was spread as tiny droplets on the surface of the subphase using an aspirator and the cleanliness was ensured by a zero reading of the surface pressure. One mg of tissue homogenate was spread as tiny droplets on the surface of the subphase using a Hamilton syringe. Isotherms were standardized as monolayers corresponding to equal weights and cell counts in all cases.

The surface pressure–area isotherms were recorded by continuous compression and expansion of the monolayer for three cycles (1 cycle = 1 compression + 1 expansion) with a barrier speed of 120 mm/min. The maximum relative area change during compression was 86.5%. The surface activity of tissue homogenates, organic phase and aqueous phase of cancerous and normal cervical tissues were evaluated as monolayers. Effect of radiation on the monolayers was also monitored.

2.7. Calculation of surface parameters

From the surface pressure–area isotherms obtained, the following parameters were calculated. The minimum surface tension (γ_min) was calculated as γ_min = γ_s − π_max, where π_max is the maximum surface pressure and γ_s is the surface tension of the subphase. The minimum surface tension is indicative of the interfacial properties of the fully compressed monolayer. Relative limiting area (A_0) and relative lift off area (A_1) are quantities, which indicate molecular packing and interaction of molecules in the monolayer. The relative limiting area was determined by extrapolating the final steep linear region of the isotherm at end compression to the % area axis. The relative lift off area was obtained by extrapolating the area at which an increase in surface pressure from the baseline value was observed to the % area axis. Another parameter of interest was the hysteresis area (ΔG), which is indicative of energy trapped in a monolayer. The hysteresis area is the difference between the free energy of compression and free energy of expansion, which were calculated from the area under the corresponding surface pressure–area isotherms.

2.8. Langmuir–Blodgett film deposition and atomic force microscopy

The monolayer at the air–water interface was transferred on to a clean mica sheet (30 × 10 × 1.5 mm), using Langmuir–Blodgett deposition technique. The monolayer was first compressed to a pre-determined target surface pressure value (20 mN/m) with the help of servo mechanism and a clean mica sheet, immersed into the subphase prior to monolayer spreading, was withdrawn from the monolayer with a speed of 5 mm/min. The transfer ratio was monitored throughout the dipping process and films having transfer ratios close to or equal to one were taken for atomic force microscopy (AFM) measurements after drying in air for two hours. AFM measurements were made at room temperature (25°C) using an optical lever microscope (Nanoscope IV, Digital Instruments, Santa Barbara, CA) and contact mode imaging. The topographic images were taken using oxide sharpened silicon nitride cantilever at a scan rate of 1 Hz.

2.9. Lipid quantification

The quantification of total phospholipid and cholesterol contents of the normal cancerous cervical tissues was also carried out in our study. The total phospholipid content was quantified by malachite green procedure. Briefly, a small part of the organic phase extracted from the tissue homogenate was digested with perchloric acid to get inorganic phosphorus from the phospholipids. To this, a solution containing malachite green (0.08% in deionized water), ammonium molybdate (5.72% in 6 N hydrochloric acid) and polyvinyl alcohol (2.32% in boiling deionized water) in the ratio 2:1:1 (by volume) was added to develop the desired color and the optical density of the colored solution was measured at 640 nm in a UV–visible spectrometer (UV 8500, Techcomp, China) using the photometric mode. The concentration of phosphorus in our lipid was determined from the calibration curve obtained by the above method using standard solutions of potassium dihydrogen phosphate having varying phosphorus concentrations. The amount of phosphorus in the lipid was multiplied by a factor 25 to get a measure of the total amount of phospholipid.

The total cholesterol content was also quantified in this study by a colorimetric method using ortho-phthalaldehyde in glacial acetic acid and concentrated sulphuric acid as the coloring agents. A known amount of the organic phase extracted from the tissue homogenate was treated with 1 ml of 3% methanolic potassium hydroxide followed by extraction with 2 ml hexane. The hexane phase was separated, evaporated to dryness and residue dissolved in 1 ml dichloromethane. To a known amount of dichloromethane aliquot, 1 ml of ortho-phthalaldehyde in glacial acetic acid was added, stirred well and treated with 0.5 ml of concentrated sulphuric acid. After 15 min the absorbance at 550 nm was measured in a UV–visible spectrometer (Lambda 25, Perkin Elmer). The concentration of cholesterol in
cervical lipid was determined from the calibration curve obtained by the above method using standard solutions of cholesterol having varying concentrations.

2.10. Statistical analysis

Twenty human cervical cancer tissues (both pre- and post-radiotherapy samples) and fifteen normal human cervical tissues were characterized in this study and for each tissue monolayer tensiometric parameters were calculated as explained earlier. The data were expressed as mean ± standard deviation. Comparisons for statistical differences were performed by Mann–Whitney test (\( p < 0.05 \)).

3. Results

Fig. 1 represents the first compression surface pressure–area isotherms of normal, cancerous pre-radiotherapy and cancerous post-radiotherapy tissue homogenate monolayers. The tensiometric profiles of the post-radiotherapy cancerous samples were in two groups. In one group (\( n = 4 \)) the mean isotherms showed a horizontal region up to 40% area of compression and was similar to that of the cancerous pre-radiotherapy (\( n = 20 \)) isotherm. In the second group (\( n = 16 \)) the mean compression isotherm showed an increase in surface pressure at about 85% area and reached a maximum surface pressure of 33 mN/m, this profile was similar to that of the normal cervical tissue isotherm (\( n = 15 \)). On comparison with the clinical radioresponsiveness at the end of the therapy, it was found that the cases where the post-radiotherapy isotherms were similar to normals were in fact clinically completely radioresponsive. The cases where the isotherms of the post-radiotherapy samples were similar to the pre-radiotherapy samples were clinically partially radioresponsive at the end of the therapy.

To account for the intra-tumoral heterogeneity in tensiometric parameters, six different sections from a cervical cancerous tissue biopsy sample have been evaluated and the coefficient of variation values were found to be less than 0.05, indicating lower intra-tumoral heterogeneity in tensiometric parameters. Histopathology analysis also revealed similar type of pathology for the cancerous tissues involved in this study. Further, literature evidence also reveals low levels of intra-tumoral heterogeneity for cervical tumors compared to other tumors like glioblastomas [11].

Figs. 2–4 depict the calculated tensiometric parameters of normal as well as pre- and post-radiotherapy cervical cancerous tissue homogenate monolayers. It is clear that all the four tensiometric parameters showed statistically significant difference (\( p < 0.05 \)) in the completely and partially radioresponsive groups. The minimum surface tension of partially responsive post-radiotherapy tissue homogenate was 1.3 times greater than that of completely responsive post-radiotherapy tissue ho-
homogenate. Similarly, $\Delta G$, $A_1$ and $A_0$ values of partially radioresponsive post-radiotherapy tissue homogenates were 6.0, 1.7 and 2.2 times less than those of completely radioresponsive post-radiotherapy tissue homogenates, respectively. Thus the tensiometric parameters as well as the isotherm shapes of cervical tissue monolayers were distinctly different in completely radioresponsive and partially radioresponsive cases. Further, no statistically significant difference (in all the four tensiometric parameters) was found between partially radioresponsive post-radiotherapy and cancerous pre-radiotherapy tissue homogenates. All tensiometric parameters of the partially responsive group were significantly different from the normal controls.

In case of the clinically responsive group all the parameters showed significant difference ($p < 0.05$) when compared to the cancerous pre-radiotherapy cases. For example, the minimum surface tension of completely responsive post-radiotherapy cancerous tissue homogenate was about 1.4 times less than that of cancerous pre-radiotherapy tissue homogenate and was statistically similar to that of the normal cervical tissue homogenate. Similarly, $\Delta G$, $A_1$ and $A_0$ values of completely responsive post-radiotherapy cancerous tissue homogenate were 3.5, 1.6 and 2.3 times higher than those of cancerous pre-radiotherapy tissue homogenate, respectively, and were closer to those of normal cervical tissue homogenate. From the above described results it can be concluded that after first dose of radiation, if the minimum surface tension of a cervical tissue monolayer is less than 47 mN/m and the hysteresis area is greater than 33 µJ then that tissue can be counted as a completely radioresponsive one. Likewise the tensiometric criteria for partially radioresponsive cervical tissues is minimum surface tension greater than 50 mN/m and hysteresis area less than 20 µJ. The sensitivity and specificity of tensiometric radioresponsiveness prediction was found to be 100% when compared with the clinical radioresponsiveness.

The aqueous and organic phases extracted from tissue homogenates were also characterized to reveal the role of lipid and non-lipid components on the radiation induced tensiometric profiles. Both completely and partially radioresponsive samples showed similar surface activity profiles for their aqueous phases before and after radiotherapy as shown in Figs. 5a and 5b, respectively. Fig. 6 represents the effect of radiation on the tensiometric profiles of organic phases of cervical cancerous tissues. It is interesting to note the shifting of tensiometric profile of the organic phase of irradiated completely radioresponsive tissues toward that of normal cervical organic phase. In case of partially radioresponsive tissues, the organic phase surface activity was not considerably affected by radiotherapy. The organic phase of completely radioresponsive cancerous cervical tissue showed tensiometric profiles with maximum surface pressures of $\sim 45$ and 31 mN/m prior and post radiotherapy, respectively, whereas the organic phase of partially radioresponsive tissue showed profiles with similar maximum surface pressures ($\sim 47$ mN/m) before and after therapy. For completely radioresponsive cancerous cervical tissues, the organic phase

![Fig. 4](image44x581to292x724) Fig. 4. Effect of radiotherapy on lift off area and limiting area of clinically partially and completely radioresponsive cancerous cervical tissue homogenate monolayers. Data expressed as mean ± standard deviation. Cancerous pre-radiotherapy, $n = 20$, normal, $n = 15$, clinically partially responsive post, $n = 4$ and clinically completely responsive post, $n = 16$.

![Fig. 5](image114x72to491x267) Fig. 5. Effect of radiotherapy on surface pressure–area isotherms of clinically partially and completely radioresponsive cancerous cervical tissue aqueous phases. Only first compression isotherms at 37 °C are shown here. For partially radioresponsive isotherms $n = 3$ and for completely radioresponsive isotherms $n = 4$. 
Fig. 6. Effect of radiotherapy on surface pressure–area isotherms of clinically partially and completely radioresponsive cancerous cervical tissue organic phases. Only first compression isotherms at 37°C are shown here. For cancerous isotherms \( n = 3 \) and for normal isotherms \( n = 15 \).

Fig. 7. AFM images of tissue homogenate and organic phase monolayers of clinically completely radioresponsive cancerous human cervical tissues. AFM images are of (2 µm × 2 µm) size. (a) Pre-radiotherapy tissue homogenate, (b) post-radiotherapy tissue homogenate, (c) pre-radiotherapy organic phase and (d) post-radiotherapy organic phase.

Minimum surface tension values before and after therapy were 26.6 ± 3.6 and 41.1 ± 2.8 mN/m, respectively, and was significantly different \((p < 0.05)\). Furthermore, the organic phase of completely radioresponsive cancerous tissues showed a minimum surface tension value similar to that of normal cervical organic phase (44.8 ± 4.0 mN/m). The organic phase minimum surface tension values of partially radioresponsive cancerous cervical tissues before and after radiotherapy were 24.9 ± 1.2 and 25.1 ± 3.1 mN/m, respectively.

The surface morphology of completely radioresponsive cancerous tissue homogenate and their organic phase monolayers was characterized using the contact mode AFM. Fig. 7 shows the topographic images (2 µm × 2 µm) of tissue homogenates and organic phases deposited on mica sheets. The non-treated (pre-radiotherapy) tissue homogenate monolayer had a mean surface roughness of 22.8 nm/µm² whereas the treated (post-radiotherapy) tissue homogenate monolayer had a mean surface roughness of 8.6 nm/µm². The non-treated organic phase...
monolayer had a smooth surface with a mean surface roughness of 3.2 nm/µm² as compared to the treated organic phase having mean surface roughness of 5.8 nm/µm². Further, in the case of organic phase images both before and after therapy stripes were observed as opposed to those of tissue homogenates.

The lipid levels in cancerous and normal cervical tissues are summarized in Table 1. The total phospholipid content of the cancerous cervical tissue was 2.5 and 1.2 folds higher than that of the normal tissue value before and after radiotherapy, respectively. Also in cancerous cervical tissue the total cholesterol was found to be 1.5 and 1.3 folds higher than the normal cervical tissue before and after radiotherapy, respectively.

**Table 1**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total phospholipid (mg/g of tissue)</th>
<th>Cholesterol (mg/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancerous pre-radiotherapy</td>
<td>4.83 ± 0.08</td>
<td>23.02 ± 0.28</td>
</tr>
<tr>
<td>Cancerous post-radiotherapy</td>
<td>2.19 ± 0.63</td>
<td>19.83 ± 0.98</td>
</tr>
<tr>
<td>Normal pre-radiotherapy</td>
<td>1.94 ± 0.04</td>
<td>15.45 ± 0.09</td>
</tr>
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*Note.* Expressed as mean ± standard deviations of three trials of quantification. Cancerous samples included in this quantification study were clinically completely radioresponsive tissues.

The tensiometric parameters were able to differentiate between partially and totally radioresponsive cancerous cervical tissues. All the isotherms in Fig. 1 showed plateaus at the starting region of compression which is indicative of gas to liquid expanded phase transition in the monolayers [12]. Tissues having lower minimum surface tensions are more closely packed and in the gel state as compared to more fluid monolayers having higher minimum surface tension [8]. Thus the fluidity of the tissue homogenate monolayers in our study can be represented as (normal = completely responsive post-radiotherapy) < (cancerous partially responsive post-radiotherapy = cancerous pre-radiotherapy).

The tensiometric profile of radioresponsive pre-radiotherapy cancerous tissue homogenates shifted towards that of normal after exposure to radiation. This may be due to the radiation induced biochemical changes in the tissues. The total cholesterol and phospholipid content of completely radioresponsive cervical tissues was found to be significantly reduced after a single dose of radiation of 2 Gy. The alteration in phospholipid content after exposure to radiation may be due to the altered phospholipase activity which is also seen in literature [13]. In cancerous cervical tissues phosphatidylcholine was found to be the major phospholipid accounting for about 30% of the total phospholipids whereas in normal cervical tissue the major phospholipid was sphingomyelin [14]. The tensiometric profile of radiosensitive cancerous tissues shifted towards that of normal (having less phospholipid than cancerous pre-radiotherapy) after irradiation in our results which can be correlated to the decrease in phospholipid content after irradiation.

In our results the tensiometric profiles of radiosensitive cervical tissues showed a rigidifying effect after exposure to radiation as seen by a lower value of minimum surface tension. Alterations in membrane fluidity by the action of ionizing radiation have been reported in several studies. Radiation induced rigidization of membrane observed can be attributed to the radiation initiated radical generation and subsequent reactions with lipid molecules of the membranes, producing peroxidation and crosslinking of membrane components by peroxidative products [15–17].

Our lipid quantification studies established a significant reduction of cholesterol and total phospholipid in the completely radioresponsive cancerous tissues after treatment with first dose of radiation. This can explain the reduced surface activity of the organic phase after radiotherapy as compared to that of the organic phase before radiotherapy (see Fig. 6a). In completely radioresponsive cancerous tissues, the change of lipid profile after radiation was towards that of normal cervical tissue and similar trend was observed in the tensiometric profiles of these tissue homogenates. However, the organic phase of partially radioresponsive cancerous tissues showed similar surface activity before and after treatment (Fig. 6b).

The aqueous phase surface activity was not significantly altered by the effect of radiation in both completely and partially radioresponsive cervical cancerous tissues, indicating that the aqueous phase components may have lesser contribution towards the radiation induced tensiometric profiles of cervical cancerous tissue homogenates. On the other hand the surface activities of tissue homogenates and organic phases showed radiation induced changes in completely radioresponsive cancerous tissues as opposed to that in partially responsive cancerous tissues. From these results one can presume that the effect of radiation in cervical tissues may be either due to the lipophilic–lipophilic (organic–organic) or lipophilic–lipophobic (organic–aqueous) interactions.

The surface roughness analysis of AFM images of tissue homogenates showed a smoothing of the tissue homogenate surface after radiotherapy in case of completely radioresponsive cancerous tissues. A rough surface indicates more disorder in the monolayer packing compared to a smoother surface. Thus pre-radiotherapy tissue homogenate was found to be more disordered than the post-radiotherapy tissue homogenate. This finding agrees well with the comparatively higher fluidity, as predicted by the minimum surface tension values, of the completely radioresponsive pre-radiotherapy tissue homogenates.

The pre-radiotherapy organic phase monolayer was smoother than the post-radiotherapy organic phase monolayer, indicating a more ordered molecular packing in it. A study by Kim et al., established an increase of surface roughness of the AFM images of mixed monolayers of DPPC and cholesterol on reducing the amount of cholesterol from 30 to 10% [18]. The relatively higher roughness of post-radiotherapy organic phase monolayer can be correlated with the higher cholesterol content in it compared to the pre-radiotherapy organic phase monolayer. In organic phase monolayers also the fluidity predicted by surface roughness values was in good agreement with that predicted by the minimum surface tension values. Thus AFM studies of tissue homogenates and organic phases also support the fact that the effect of radiation in completely radioresponsive cervical
cancerous tissues may be mainly due to the radiation induced alterations in the lipid profiles.

5. Summary

Our study revealed that the statistically different tensiometric parameters have the potential to predict the radiosensitivity of the cervical tumours. All the four tensiometric parameters were potential markers of partially and completely responsive post-radiotherapy cancerous tissues. From our results, the following tensiometric criteria for prediction of radioresistance emerge. After first dose of radiation, if the minimum surface tension is greater than 50 mN/m and hysteresis area is less than 20 µJ those tissues will be classified in the partially radioresponsive category. Similarly for completely radioresponsive tissues, the minimum surface tension value will be less than 47 mN/m and the hysteresis area will be greater than 33 µJ. The classification based on these tensiometric profiles after a single dose of radiation was in agreement with the clinical radioresponsive ness data collected at the end of radiotherapy. The clinically radioresponsive tissues show a decrease in minimum surface tension on application of radiation. This is due to changes in the surface activity of the organic phase of the monolayers. Radiation led to a decrease in the total phospholipid and cholesterol levels in responsive tissues and a lower surface roughness and organization of the monolayers. The sensitivity and specificity of the tissue monolayer technique was found to be 100% based on the clinical responsiveness evaluated at the end of the treatment after the entire dose of radiation. Moreover, the Langmuir tissue tensiometry is simple and accurate. It requires only half an hour for evaluation of a sample and is easily translatable to the clinical laboratories. Some of the limitations of the present study are its small sample size and lack of follow up data on disease free survival. Nevertheless, the results are promising and show very less intra-tumoral coefficient of variation. Further studies are warranted for a wide spectrum of cancers with large sample sizes for validation of this promising technique.

References