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Static magnetic field effects on the collagen molecules; a possible biophysical approach to control cell metastases

Maryam Akbari[1], Hamid Mobasheri[2, 3, *], Luciana Dini[4], Aida mahdipour[5]

[1]Laboratory of Membrane Biophysics and Macromolecules, Institute of Biochemistry and Biophysics, University of Tehran, Iran, maryamakbari.1369@ut.ac.ir.

[2]Laboratory of Membrane Biophysics and Macromolecules, Institute of Biochemistry and Biophysics, University of Tehran, Iran, h.mobasheri@ut.ac.ir

[3]Institute of Biomaterials of University of Tehran and Tehran University of Medical Sciences (IBUTUMS), Tehran, Iran, h.mobasheri@ut.ac.ir

[4] Department of Biology and Biotechnology C. Darwin, Sapienza University of Rome, Rome, Italy, luciana.dini@uniroma1.it.

[5]Laboratory of Membrane Biophysics and Macromolecules, Institute of Biochemistry and Biophysics, University of Tehran, Iran, mailto: mailto:aida.mehdipour@ut.ac.ir.

[*]Corresponding author

Abstract

Type I collagen is among the major extracellular proteins that plays a significant role in cell growth, mutility, migration, proliferation, differentiation, and inflammation. Collagen is involved in the development and progression of many tumors through various signaling pathways and cytokines. The present study investigated the effect of static magnetic field (SMF) 300 mT on the structure and molecular properties of Type I collagens.

The purity of collagen was confirmed by SDS-PAGE, its secondary structure properties was revealed by Circular Dichroism (CD) and chemical bound properties by Fourier

Transforms Infrared (FTIR) spectroscopy. Also, the Physico-chemical characterization of the collagen was studied using Zetasizer light Scattering and Rheological assay.
Our results revealed that The pH of the treated collagen solution increased by while the Zeta potential decreased. The rheological experiments revealed changes in the viscoelastic behavior of collagen due to exposure to SMF 300mT making it less elastic. However, the secondary structure of collagen molecules did not change significantly and the typical positive and negative remained intact.
Accordingly, the SMF is considered as a promising biophysical means for targeting type I collagen to control and manipulate cell metastases, motility and division as a prevention and treatment means to tackle with cancer.

Keywords: Biophysics, Cancer, Static magnetic field, Type I Collagen

1- Introduction

Type I collagen is the main protein of the extracellular matrix and consists of tropocollagen fibrils (300nm in length) made of three polypeptide chains arranged in a triple-helix, forming collagen fibers. The collagen triple-helix consists of a repeating (Gly-X-Y)n sequence in which, very frequently, X and Y correspond to proline and hydroxyproline, respectively [1]. The triple-helix and its arrangement into the fibre are stabilized by two different types of hydrogen bonds. More precisely, direct hydrogen bonds between the N–H group of glycine of one chain and the C=O group of X residue of another chain, or indirect hydrogen bonds involving water molecules [2]. Consequently, water is crucial for maintaining collagen structure [3].

Collagen type I is the most important collagen in cancer because the expression levels are higher in malignant tumors, and has the potential to become a new tumor marker and aid the diagnosis of benign and malignant tumors and metastasis [4].

Clinical application of natural and man made SMF has shown to have positive influence on a broad range of physiological processes and can be considered as potential candidate for therapeutic purposes. Consequently, here, SMF has been developed as an alternative,

noninvasive and safe therapeutic tool for manipulation of the structure of collagene molecules in order to use them for control and if possible prevent cell devision, migration , proliferation and adhesion addressed by others [5-7].

2- Methodology

2-1 Collagen extraction

Collagens were extracted from the tendons of Wistar Hannover male specimens [8]. All animal experiments were conducted according to the ethics and concepts defined by the Animal Experimentation Ethics Committee of the University of Tehran, approved by the Iran NationalCommittee for Ethics in Biomedical Research (IR.UT.SCIENCE.REC.1400.012). The extracted collagen was adjusted to pH 3.5. The purity and identity of the extracted collagen were assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2-2 SMF application on collagen molecules

The collagen molecules were exposed to SMF using tailored chambers made of Plexiglas equipped with NdFeB neodymium permanent magnets to induce homogeneous SMFs with intensities of 300 mT applied by two parallel magnets (Fig. 1).

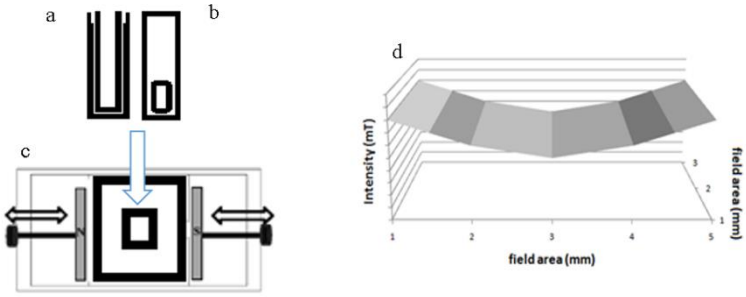

Figure 1: SMF applier chamber. Showing the cuvette (a) and the cuvette holder (b) in place between two neodymium magnets in the designed chamber (c). The schematic design of the homogeneity of permanent magnets was shown using a gauss meter (d).

Effect of the SMF on the secondary structure of collagen

The secondary structures of type I collagen molecules were investigated by a Circular Dichroism Spectropolarimeter (J-815, Jasco Corporation, Japan). The chemical composition of intact and SMF 300 mT treated type I collagen samples was studied by a Fourier Transform Infrared Spectroscopy (FTIR) instrument (Nexus470, Thermo Scientific).

The effect of the SMF on surface charge

A zeta potential analyser (Zetapals, Brookhaven Instruments Corporation) was used to recognise the surface charge of type I collagen after exposure to the SMF 300 mT.

3- Results

3.1 The purity and molecular weitght of the extracted protein samples checked by SDD PAGE revealed its purity and quality (Fig. 2).

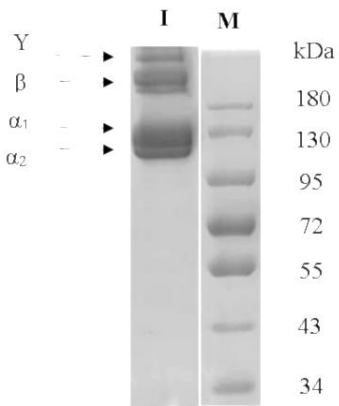

Figure 2: SDS-PAGE nalysis of the purity of type I collagen protein. Runing collagen samples on SDS-PAGE, 8% acrylamide gel and its staining with Coomassie Brilliant G-250 showed the purity of the extracted molecules The well I shows the presence of alpha 1, alpha 2, beta and gamma type I collagen. The standard markers with Mw of 34 to 180 kDa were loaded in the well M.

3.2 Effects of 300 mT SMF on the secondary structure of collagen

Characterization of the triple helix structure of type I collagen molecules before and after exposure to SMF 300 mT for 5, 10, 15, and 20 minutes was carried out by means of CD spectroscopy, which utilized the differential absorption of left and right handed circular polarized light in an asymmetric environment to assess secondary structure and the results are shown in Fig.3. The CD spectra displays a characteristic positive peak at 218 nm that corresponds to the triple-helical structure followed by negative peak at 198 nm for polyproline type II (PP II) structures **.**

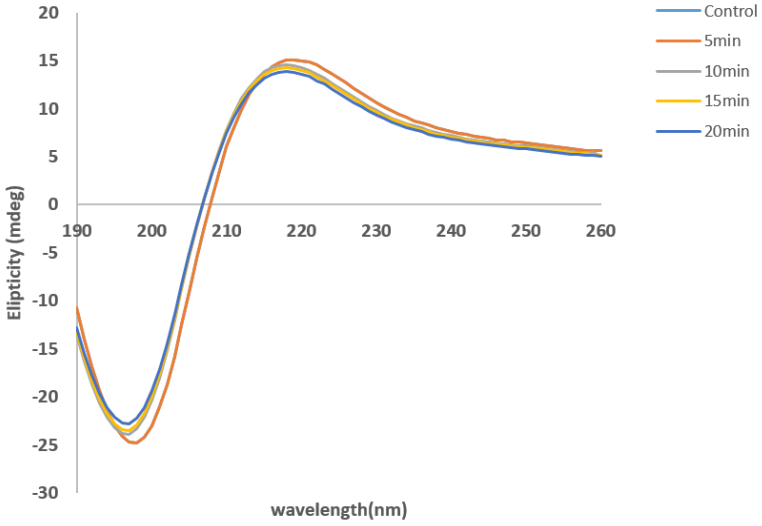

Figure 3: CD spectrum of Type I rat tail collagen in the absence and presence of 300 mT magnetic field.Type I collagen (control) shown in blue and the SMF exposed ones for 5 minutes in orange, and for 10, 15 and 20 minutes in gray, green and dark blue respectively. A change in the intensity of the spectrum was observed in the presence of the SMF. Also, there was no change in the positive and negative peaks of the collagen structure in the presence of the SMF at the mentioned times.

3.3 Effect of pH on the secondary structure of type I collagen

The CD spectrum showed the changes in the secondary structure of type I collagen at different pH. The pH of extracted collagen was set to different pH to identify the pH effect on their secondary structure spectrum (Fig. 4).

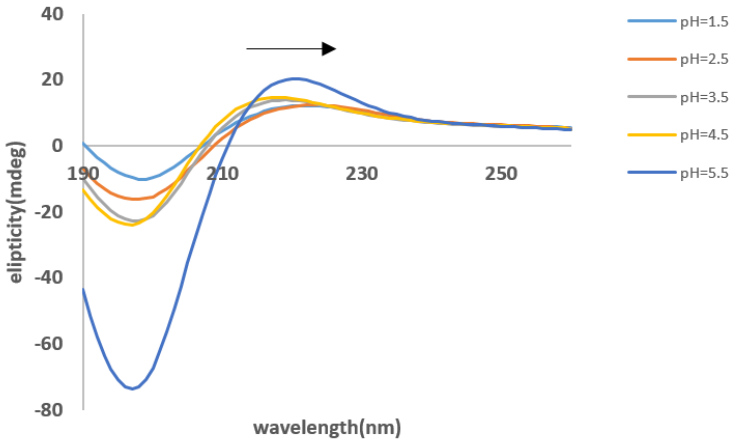

Figure 4: CD spectrum of type I collagen at different pH. The pH of the extracted collagen solution was set to 1.5-2.5-3.5-4.5-5.5 before the CD spectrum was taken.

3.4 Effects of of 300 mT SMF on the chemical composition of type I rat tail collagen in at different inqubation times.

ATR-FTIR spectroscopy was used to study the preservation of the structure of type I collagen (0.3 mg/ml) as well as the changes of its functional groups after applying a SMF 300 mT for 5-10-15-20 minutes. As shown in (Fig. 5), the preservation of the collagen structure in the presence of magnetic fields was observed in the wave numbers corresponding to amide I, II and III.

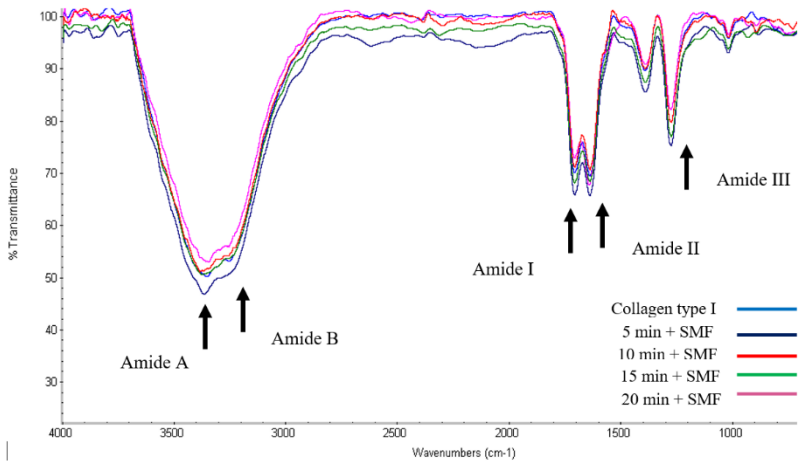

Figure 5: FTIR spectrum of Type I collagen in the presence of 300 mT magnetic field. Type I collagen (control) blue spectrum, dark blue spectrum exposed to the field for 5 minutes, red spectrum exposed to the field for 10 minutes, green spectrum exposed to the field for 15 minutes and the pink spectrum is exposed to the field for 20 minutes. The wavenumbers cm^{-1} 1652, cm^{-1} 1548 and cm^{-1} 1240, respectively, were observed for amide I, amide type II and type III of collagen type I structure in the presence of magnetic field at the mentioned times.

3.5 Effects of 300 mT SMF on the surface potential of Type I collagen

The zeta potential of the collagen molecules in the presence and absence of 300mT SMF was measured to determine possible changes in the surface potential and the repulsion or electrostatic attraction of collagen molecules (Table 1-3). The zeta potential of 300 mT SMF treated collagen samples was measured with three repetitions. Type I collagen solution with a concentration of 0.3 mg/ml and pH=3.5 was calculated three times.

Table 3-1- Zeta potential of type I collagen solution in the presence of magnetic field

Collagen (control)	300mT SMF treated Collagen (5 min)
+5	-8.3

4. **Conclusion**

Exposure to SMF at different intensities, times and directions can have different effects on the biological systems. Type I collagens play vital role in keeping the structure and function of the extracellular matrix as well as in cellular processes including tissue repair, immune responses, cellular communication, resistance and cell mechanics that matters in normal cells and deviated in malignant ones. The surface potential and charge distriution of collagen molecules are sensitive to their medium pH. On the other hand most of activities of cancer cells involves proper electrostatic interaction of collagen molecules with themselves as well as the corresponding molecules to fulfil their metastases, devison and so on. Here, we have shown that exposure to SMF can decrease collagen medium. Thus, SMF has the potential of changing collagen surface chages by which different machinery of cancer cells can be ceased, or enhanced. In other words SMF exposure can control the acidic microenvironment within a tumour that promote migration, invasion and metastasis of cancer cells. Consecuently, taking the biophysical approach presented here, have promising outcomes applicable in prevention, control or treatment of cancer cells at different stages.

Acknowledgement

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