Fibroblasts Cell Number Density based Human Skin Characterization at THz for In-body Nanonetworks

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Abstract

This paper presents the investigation of the electromagnetic properties of human skin tissues using Terahertz Time Domain Spectroscopy (THz-TDS). The material parameters i.e., refractive index and absorption coefficient are extracted for artificially synthesized skin cultured using fibroblast cells and collagen type I reagent. The increase in cell count number by 200% will cause a distinctive decrease in refractive index and absorption coefficient values. In addition to material parameters, in-body channel parameters i.e., total pathloss and molecular noise temperature of the skin are also calculated. The results show the dependency of channel parameters on molecular features and hydration level of the skin. Such findings will path the way for more rigorous THz channel analysis and network modeling to be applied for body-centric nano-communication specifically in the bioengineering domain.

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1. Introduction

Nanoscale communication as proposed by the IEEE P1906.1 framework must verify to the basic elements of communication theory, including transmitter, receiver, medium and message carrier [1]. The incorporation of nanotechnology to various research domains such as biomedical, imaging, electronics and material fabrication has lead to the development of new generation healthcare applications. Nanoscale communication is a recent addition to the realms of nanotechnology aiming to broaden the capabilities of communication technology. The applications of nanoscale communication have been extensively discussed in literature [2,3] and subsequently various communication schemes have been proposed [4,5]. Amongst the pool of applications ,

in-body nanonetworks as a health diagnostic utility has garnered immesed popularity in the past decade [3]. The apothesis of this technology is the ability to transpire intricate information of the human tissue by means of electromagnetic (EM) waves. Admist the proposed mechanisms for nanoscale communication, EM communication is considered as the most reliable and controled way of realising the tangibility of in-body nanonetworks [6,7]. Nanoscale EM communication (or nanocommunication) is an efficient contrivance of information exchange between nanoscale devices (or nanosensors), usually fabricated with graphene and other nanomaterials. For instance, graphene plasmonic miniaturized antennas are envisoned to implement nanoscale EM communication among nanosystems resonating in the THz band with a higher radiation efficiency in comparison to their metallic counterparts [8]. Assimilating from the heirarchy of any communication system (including the human body) , characterization of channel medium is of utmost importance and the same applies to in-body nanonetworks. The research published in this area [6] investigate channel capacity of in-body nanonetworks in the lossy air medium. This work substantiates the ability of THz communication to offer more than 1 Tb/s of physical transmission rate and propagation distances of the order of few tens of millimeters . Channel propagation models for free space for nanonetworks have been proposed based on radiative transfer theory. The results for both pathloss and molecular noise temperature were published using HITRAN database for atmospheric gas molecules. It has already been established that operation of in-body nanonetworks is only relevant for short transmission range [9]. In the light of these results, nanoscale EM communication proposed for healthcare applications, needs a thorough analysis of manifested channel medium, which is the human tissue. In this paper, study is performed on the material properties of human skin in addition to in-body nano-network channel parameters within the THz range of frequencies. Skin makes an interesting choice of study, since it is the most abundant tissue present and offers dynamic analysis due to its varied hydration levels throughout the human body. The variation in tissue hydration influences the texture and dimension, which in turn could affect the material properties. From a biology perspective, skin is a complex layered structure. However, for simplicity it can be divided into three main visibly defined layers : stratum corneum, dermis and subcutaneous fat . Out of these, dermis is the thickest and most abundant in water, hence resemble to properties of that of free water [10-13]. Its main constituent is collagen, a fibrous protein rich in glycine and proline, that is a major component of the extracellular matrix and connective tissues [14]. Hence, collagen is the most widely used for medicinal purposes such as, treating tissue burns , drug delivery system, controlling material for transdermal delivery and as surgical suture [15]. Many natural polymers and their synthetic substitutes are used as biomaterials, but the unique characteristics play an important role in the formation of tissues and organs. For instance, Collagen when compared with Integra Derma, Allograft and Opsite (systhetic substitute) exhibits superior bio-compatibility and contitutes more than

30% of skin tissue. It forms extra fine fibers through its self-aggregation and cross-linking capabilties. Whereas other systhetic tissues are mainly polymer like cultured from non-biological molecules.

To the best knowledge of authors, the biological modelling and characterization of collagen is at its infancy and needs an in-depth investigation. The dielectric behavior of tissues have been extensively studied from microwave to optical range of frequencies for excised animal and complex biomolecules [16,17]. EM spectrum ranging from 0.1-10 THz bridges the gap between microwave and optical frequencies and has growing number of applications as mentioned above. Its unique spectral features and sensitivity to water, makes it a viable technique for investigating material properties of tissues, skin layers and hydration dynamics [18,19].Some papers [13,20, 21,28,29] have covered wide range of domains that investigate cell-protein interaction of collagen, hydration dynamics and THz TDS of artificial skin. The results published in open literature for optical properties of collagen are limited and differ in methodology. However, the material extraction techniques remain similar in most of these papers. For instance, in paper [20], Integra derma regeneration templates divided as monolayer and bilayer were investigated for extraction of THz material parameters. Further, the data was recorded for both hydrated and dehydrated samples. The refractive index values for dehydrated monolayer and bilayer are 1.2 and 1.4 respectively. The author also experimented the hydrated samples by varying their saline concentration but no material parameters were reported. Hence, it becomes rather complex to verify any change in hydration or dehydration level of these samples. Work done by A.G. Markelz [21], focuses on THz TDS of DNA, bovine serum albumin and collagen in the working range of 0.1-2 THz. While providing detailed analysis of other biomolecules, the work on collagen is again sparse. Experimental evaluation was done keeping in mind the humidity levels, which is important as THz wave is highly absorbed by water. Only the absorbance of collagen was reported in this paper, however it does provide some insight to hydration dependence of collagen. Most protein molecules including collagen have been evolutionary selected to have biological activity in aqueous environments. In paper [22], protein hydration shell and its role in collagen recognition was investigated. A way to generalize the properties of these tissues is asserting water as the topological basis for all skin. There is no denying in the fact that different tissues have different water hydration level, evidently age also plays an important role. In this paper, characterization of collagen (i.e., material parameter extraction) is performed using THz TDS by varying the cell number density. The results demostrated in this paper, enable us to develop channel parameters - pathloss and molecular noise temperature, for actual human tissue. Authors aim to establish a comprehensive understanding of collagen structure and construe these novel findings to develop THz channel propagation

models. The rest of the paper is organised as follows : section II ennumerates the experimental work done for synthesizing and characterizing collagen. Basic operation and techniques of THz TDS and biological modeling are explained in the subsections. Results for both absorption coefficient and refractive index are presented in section III. The channel parameters are extracted and discussed for collagen samples in section IV. A summary of work done is given in section V.

2. Methodology for Culturing and Measuring Collagen

2.1 Biological Modeling of Collagen Gels

Collagen, in its primitive form is a complex isotropic network of tangled fibrils and restraining a randomly structured matrix of highly hydrated protein compounds [14]. The molecular arrangement into fibrils is stabilized by the formation of covalent cross-links, which finally contribute to the mechanical resilience of collagen fibrils. In this work, rat-tail type I collagen is used as a base reagent for gel preparation (Fig. 1(b)). Type I is the most abundant of the collagens and naturally occurs in tendons, skin, ligaments and many interstitial connective tissues. Collagen reagent together with human fibroblasts cells forms the two vital components of the gel.


(a) Collagen   (b) Fibroblast cells
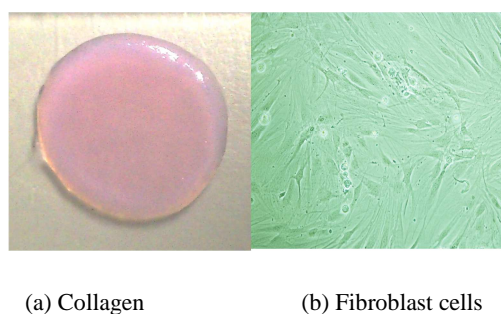
Fig. 1 (it was 3 actually) (a) Artificially synthesized collagen layer at the Blizard Institute, QMUL & (b) Fibroblasts cells assisting the growth of collagen samples.

The fibroblast cells (Fig.1 (a)) suspended in Foetal Bovine Serum (FBS) containing essential nutrients and growth factors. In addition, the gels are supplemented with concentrated Modified Eagle's Medium (MEM) – which also contains a balance of nutrients for feeding the fibroblasts. In order for the gels to set, a small quantity of sodium hydroxide is added drop wise to the collagen-fibroblast mix, until the pH indicator in the MEM turns from orange to pink. The gels are then incubated (5% CO_2) for gelation at $37^{\circ}C$ for approximately 45 minutes. Collagen gels were prepared in this study by varying the number of fibroblast cells and fixing the concentration of collagen reagent to 8 parts of 3.8 mg/ml. The cell number

was estimated while observing them in a standard light microscope using a haemocytometer. The aim is to investigate any variations in material parameters due to the differences introduced in the composition of collagen gels. Once, the initial gelation has taken place, their diameters were measured to observe any signs of contraction. They were allowed to contract to their maximum value, by keeping them in an incubator for a week. The diameter was measured again. Contraction of these gels is an important phenomenon, since the interaction of fibroblasts with collagen solution affects the thickness and overall water mass of the samples [23]. Since, it has been established that THz radiation is sensitive to changes in hydration level, it was expected to observe some unique difference in material parameters – refractive index and absorption coefficient due to the change in number of cells and volume of collagen.

2.2 Experimental Setup of the THz Time Domain Spectroscopy

Pulsed based THz spectroscopy generates and detects EM pulses and the transmitted information is taken in the time domain. Generation and detection can be done via photoconductive antenna and an electro-optic crystal. Photoconductive (PC) antenna utilizes a low temperature GaAs switch because of its unique properties such as carrier lifetime, large resistivity and good carrier mobility [24].
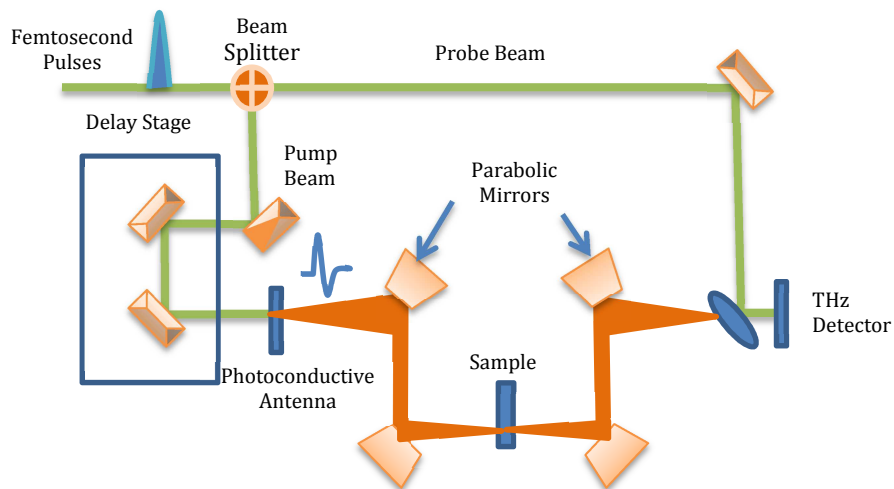
Fig 2 (it was 1 actually) Schematic Of THz-TDS [27]

The generated pulses have a wavelength suitable to the bandgap of semiconductor and hence shorten the PC switch. The THz signal produced by such mechanism is broadband consisting of wide range of frequencies, typically 0.1-1.5 THz when investigating collagen samples. These pulses are focused with reflective optics, transmitted through the sample in a holder and reach the detector system. A mechanical delay line or a translation stage introduces the time delay between generated and detected pulses. A standard THz system consist of a femtosecond (fs) laser source - Ti:Sapphire set to 800 nm wavelength and peak power of 1 W. It produces train of pulses at a repetition rate of 80 MHz and duration of 100 fs. The beam splitter divides the incoming radiation in two: pump and probe beam for coherent generation and detection of THz radiation. The intensity of generated THz radiation depends on the amount of incident beam and thus the splitter allows majority of pump beam to reach the emitter. The delay stage has maximum travel distance of 15 cm and provides a cross-correlation measurement of THz waveform. The delay line is mechanically controlled and for each sampled point, a different delay position is assigned. THz emitter is low Temperature (LT) GaAs photoconductive antenna with a biased voltage of 200 V and a gap size of approximately 0.5 mm, which makes the laser beam positioning easier. The femtosecond pulses are fed to this antenna with activation energy greater than the bandgap of LT-GaAs (1.43eV). The generated photocarriers results in pulsed photocurrent, which is directly proportional to the THz field.
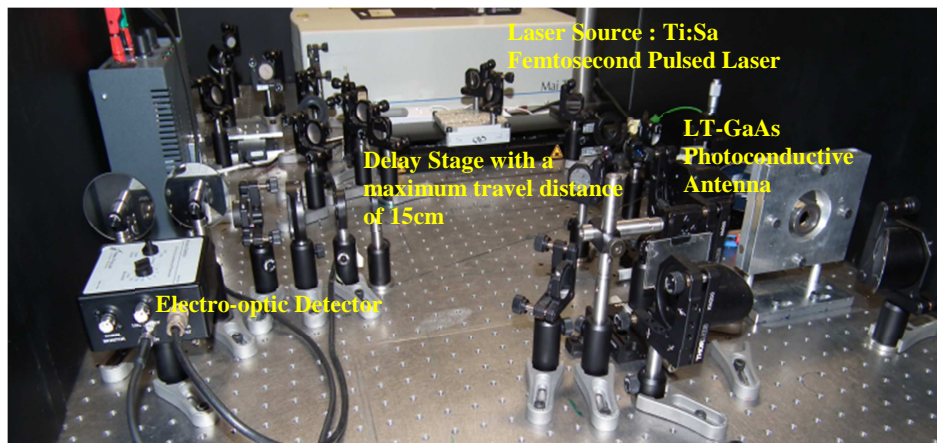
Fig.3 (it was 2 actually) THz-Time Domain Spectroscopy in transmission mode at Queen Mary University of London (QMUL)

A ZnTe crystal is employed as an electro-optic detector with thickness of 2 mm, which allows enough interaction length of probe beam and THz wave in the crystal [25]. The system is operational in transmission mode with a typical resolution of around 14.6 GHz. The radiation is focused on the sample

with a help of aluminum coated off-axis parabolic mirrors of beam diameter, 2 mm. The transmitted wave without the sample is the reference data of either free space air or a transparent material. In this case, for reference Polymethylpenthene (TPX) polymer slabs of 2.71 mm thickness is used as it has a low-loss refractive index 1.46 and absorption which is, less than 1 cm^{-1} [26]. The sample is measured by wedging collagen gels between TPX slabs mounted in a bruker liquid cell with a polytetrafluoroethylene (PTFE) spacer of thickness 200 μm. A lock-in amplifier modulates the THz signal and records the detected signal. The time domain data is recorded on the computer for air, TPX and collagen gels remotely using customized LabView® program.

2.3 Material Parameter Extraction Theory

To evaluate both phase and magnitude, Fast Fourier Transform (FFT) is applied on the data. The measured time domain signal for the different samples (collagen by varying cell concentration), air and TPX are shown in Fig. 4 and it can be seen from the figure that the biological sample is highly attenuated. The signal level attenuation of biological sample can be compared with air and TPX, where the peak intensity for collagen is reduced to 5mV for 500k cells and similarly for 300k and 100k. The oscillations and attenuation in the data is due to presence of water vapours in the atmosphere. The refractive index is calculated using the following equation [27]:

$$n(\omega) = 1 + \frac{c[\varphi_{samp}(\omega) - \varphi_{ref}(\omega)]}{\omega * d_{samp}(\omega)} \tag{1}$$

where $\varphi_{samp}(\omega) - \varphi_{ref}(\omega)$ is the phase difference between the sample and reference, corresponding to the shift in time domain. The thickness of the sample is given by d_{samp}, which is fixed to 200 μm with the help of a spacer in this study and c is the speed of light. The absorption coefficient is calculated using equation [27]:

$$\alpha(\omega) = -\frac{2}{d_{samp}} + ln\left[\frac{|E_{samp}(\omega)|}{T(\omega) * |E_{ref}(\omega)|}\right] \tag{2}$$

where $T(\omega) = \frac{4n(\omega)}{(n(\omega)+1)^2}$ is the transmission coefficient and $|E_{samp}(\omega)|$ and $|E_{ref}(\omega)|$ represents the magnitude of sample and reference in the frequency domain i.e., Fourier transform of the THz signal. The

transfer function $H(\omega)$, is defined by equation (3). It is the ratio of signal transmitted through the sample to that of transmitted without the sample. Transmitted signal without any sample is usually air or a material transparent to THz radiation. It is used as a tool to extract material properties of the given sample in the frequency domain. The above equations (1) and (2) are the result of argument and logarithm of transfer function equation.

$$H(\omega) = T(\omega) * \exp\left[-\alpha(\omega)\frac{\omega * d_{samp}}{c}\right] * \exp\left[-\frac{j(n(\omega)-1)(\omega * d_{samp})}{c}\right] \tag{3}$$

The subsequent section in the paper will use the above equations to determine the refractive index and absorption coefficients of the samples.
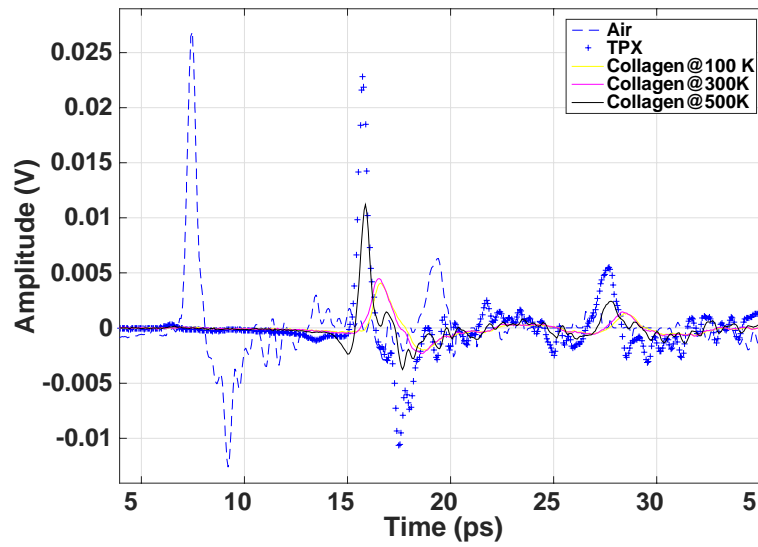

Fig. 4 THz transmission through reference plate TPX, air and cultured collagen samples of varying (1x10⁵, 3x10⁵ and 5x10⁵) number of fibroblasts cells with stock solution concentration fixed.

3. Measured Material Parameter

Sensitivity of THz radiation is dependent not only on the amount of water present but also the way in which the layers associate with water. In addition to water dynamics, the number of fibroblast cells seeded specifically for artificial skin samples, also plays an important role both in hydration level and thickness. The impact is not direct, but still is a necessary parameter to be considered while investigation optical properties of

such structures. To understand the influence of number of fibroblast cells, it is necessary to review the fundamental mechanism involved in formation of collagen gels. In many types of connective tissue such as dermis layer of the skin, the matrix-secreting cells are called fibroblasts. In 1990, P. Rompré and group [28] proposed a Box-Behnken analysis to study the effects of the collagen and fibroblast concentrations on gel thickness. The results presented verify that with increasing number of fibroblasts cells with respect to low collagen reagent concentration, the contraction increases and hence diameter of gel decreases. In the same line of work and keeping our focus on THz spectroscopy of artificial skin, the subsequent section provides details on sample preparation, cell number variation and material parameters. Referring to section [2.3], the same extraction algorithm was used to evaluate the refractive index and absorption coefficient of the samples (Fig. 5 and 6). Table II provides cell number variation and final diameter of the samples after contraction. It can be seen from the table that with increasing number of fibroblasts cells the final diameter of the samples decreases. This highlights the fact that the capacity of cell compaction of collagen fibrils, leads to reduction in volume referred to as lattice contraction. The contraction is preceded by cell-generated force that reduces the water mass between collagen fibres [23].
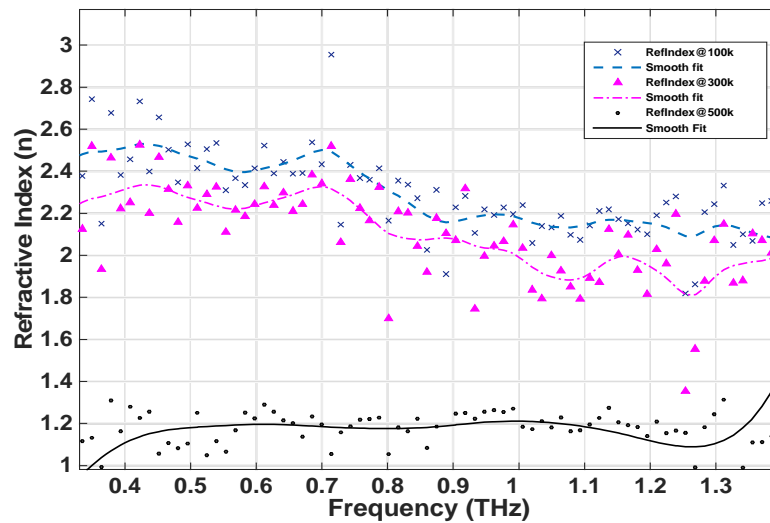

Fig.5 Refractive Index ranging from 0.5-1.3 THz illustrates that with increasing frequency the value decreases. The sample was embedded with 100k, 300k and 500k fibroblast cells. For samples with 100k and 300k, the value of refractive index is 2.2 and 2 respectively at 1 THz. However, for sample with 500k cells the value decreases to 1.2 highlighting the dynamics of collagen protein and water molecules. The sample thickness was constant at 200 μm through out the THz-TDS measuremen

Table II

Variation in cell numbers and collagen contraction

Sample	Cell no. (k=1000)	1 week old Diameter (cm)	2 weeks old Diameter (cm)	Contraction (approx.)
Coll1	100k	2.5	2	~0.5,
Coll3	300k	1.8	1.6	0.2,
Coll5	500k	1.6	1.5	0.1

In this work, the thicknesses of the samples were deliberately kept constant so as to avoid any variation in refractive index values due to change in thickness. At 1 THz, the refractive index of the samples from 100k to 500k decreases consistently (as shown in Fig 5). This suggests that concentration of water molecules do play a definitive role in changing the intrinsic nature of collagen. Sensitivity of THz radiation is dependent not only on the amount of water present but also the way in which the layers associate with water. The collagen sample (Table II, coll1) with less number of fibroblast cells demonstrates refractive index values closer to that of water [13]. Similarly, absorption coefficient values (Fig.6) are affected and decreased sharply for collagen with 500k cells. Other factors that could affect both material properties are atmospheric water vapours, gradual dehydration of samples (while performing the measurements) and etalon reflections. The first two scenarios can only be achieved in an ideal surrounding and later need further analysis. The presented value of absorption coefficient for Figure 6(a) and 6(b) are higher due to high water contents in the tissue and less contraction of cells. Maximum absorption coefficient can be measured in transmission mode by the dynamic range (DR) of the system. In this work, DR is expected to vary with the sample and is between 15-20 dB. As DR approaches its maximum value, the noise increases rapidly and any absorption coefficient features after this are invalid. The working frequency for Fig (6(a) and 6(b)) is from 0.8 – 1.4 THz where for Fig. 6(c) it is 0.4-0.9 THz. For better understanding, the noise level and irrelevant absorption coefficient peaks are truncated from the results. The absorption coefficient values for coll1 and coll2 at 1 THz are 72 cm^{-1} and 95 cm^{-1} whereas for coll3, the value sharply decreases to 40 cm^{-1} at 0.8 THz. For coll3, the noise is high since absorption coefficient rolls-off after 0.9 THz and then sharply increases.
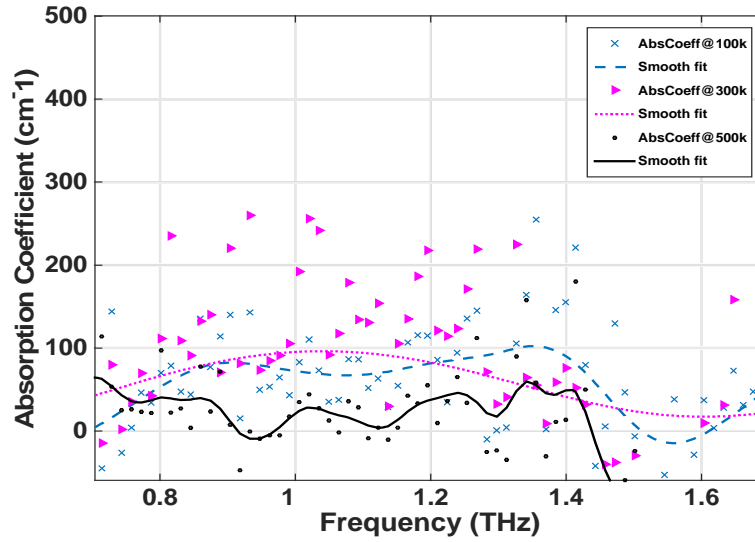
Fig. 6 The absorption coefficient calculated for 100k, 300k and 500k cells; the extra water outside the sample was carefully removed without excessively dehydrating the samples. The measured absorption coefficient is dependent on the hydration level and structure of collagen. The atmospheric water molecules in form of vapours also affect these values.

4. Pathloss and Noise Temperature Calculation

In this section, measured refractive index and absorption coefficient values are used to calculate pathloss and molecular noise temperature underlying skin as the channel medium. Both the channel parameters as function of frequency and distance are illustrated in Fig. 7 and 8. Pathloss is shown in Fig. 7 for different cell number density.
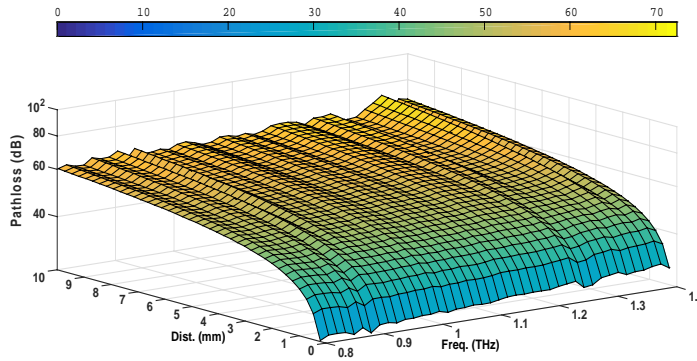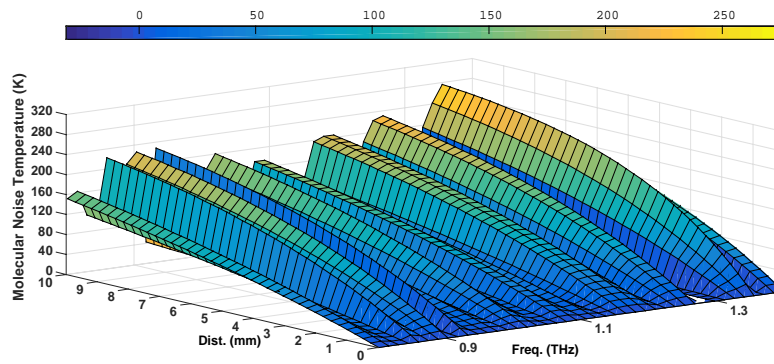

(a) Coll1 – 100k cells
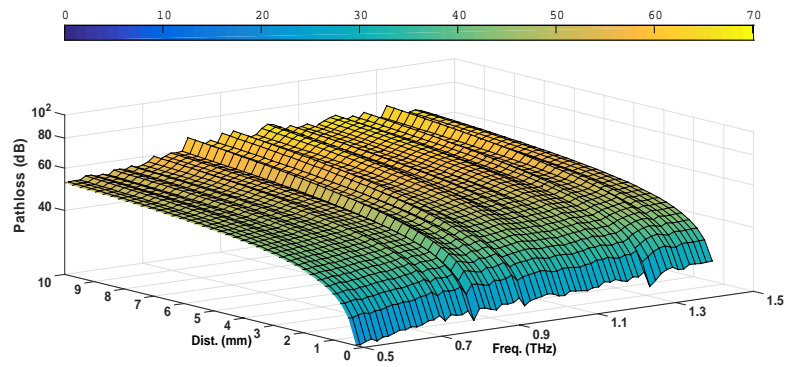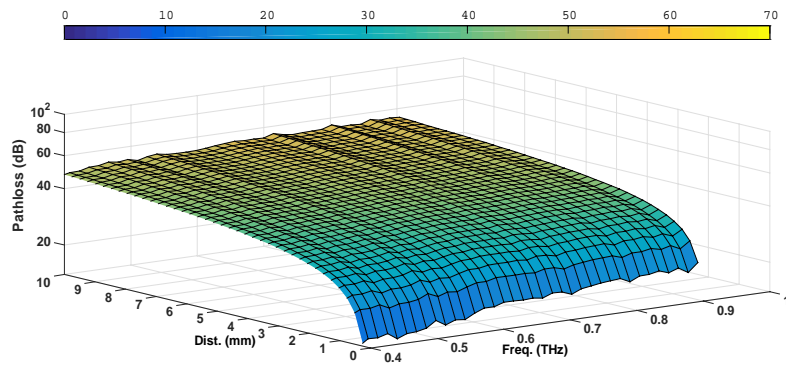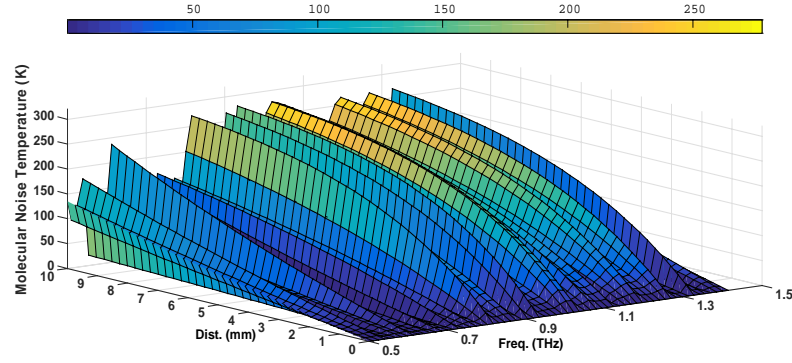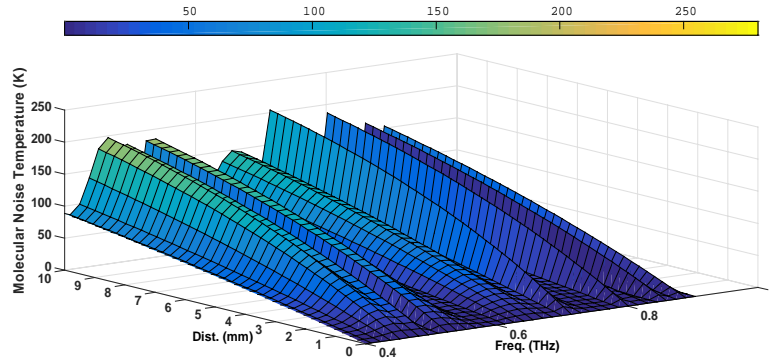
(b) Coll3 – 300k cells

(c) Coll5 – 500k cells

Fig. 7 Total pathloss as a function of frequency and distance for three different cell number densities.

(a) Coll1 – 100k cells

(b) Coll3 – 300k cells

(c) Coll5 – 500k cells

Fig. 8 Molecular Noise Temperature as a function of frequency and distance of varying cell number density.

In paper [6] it was highlighted the total absorption depends on the number of molecules that propagating wave encounters. Although, in a real human skin one expects to observe multiple varying features such as hydration level, saline concentration, collagen fibre alignment and cells number density. The applied variation in fibroblast cell number demonstrates one of the important features of human skin conformation. It can be seen from Fig. 7 (c) that the pathloss at 0.9 THz is much lower than Fig. 7 (a) and (b) due to increased cell number density and reduced water mass of collagen. As illustrated in fig. 8 (a-c), variation in cell number density affects the molecular noise temperature keeping skin as the channel medium. For transmission distance up to 1mm, the noise is high for samples Coll1 (fig.8a) and Coll3 (fig.8b), whereas for Coll5 (fig.8c) the value steeply decreases. This is due to the fact that with increasing cell number density, the total water mass (hydration level) of collagen samples decreases. The results for both pathloss and molecular noise temperature

demonstrate high dependence on molecular features and hydration level of the skin.

5. Conclusion

The complexity of skin as a channel medium is addressed in this paper by varying the fibroblast cell number density. The channel parameters depend on intricate molecular features of the human skin, which needs to be considered for in-body nanonetworks. The cell number density affects the water dynamics in the skin tissue and THz Time Domain Spectroscopy is a suitable technique to investigate the resulting structural variations. Collagen acts as scaffolding and hence it is an important protein structure for human skin. Refractive and absorption coefficient value were obtained for varying cell number density keeping the thickness of the sample constant. Increase in Fibroblast cell number density leads to collagen gel contraction and hence alters its molecular composition. This indicates to one of the important variable factors in actual human skin. Also, the water dynamics of the samples was investigated. The refractive index and absorption coefficient value decreases with increased cell number density due to reduced water mass of the samples. The overall change in conformation of the collagen samples was included while calculating total pathloss and molecular noise temperature of THz wave channel. Results for collagen with higher cell number density show better channel parameter for short transmission distances and also shows that the concentration of collagen and fibroblast cell number directly affects the water dynamics of skin. The results demonstrated in this paper, will be a stepping-stone for optimizing channel parameters of in-body nanonetworks along with a detailed analysis of skin composition.

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