Ultrasound enhancement of optical clearing of *ex vivo* skin

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Ultasound enhancement of skin optical clearing *ex vivo* in combination with chemical enhancement is demonstrated. Skin permeability was evaluated at application of ultrasound and without it. The efficiency of optical clearing of skin samples at different wavelengths were estimated.

MATERIALS AND METHODS

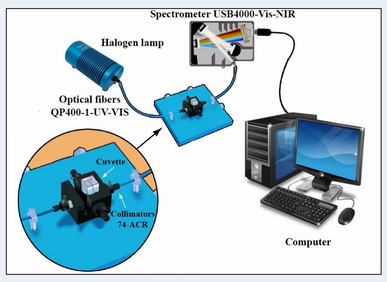
Measurement of collimated transmittance

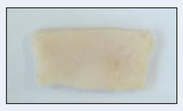
Samples: *ex vivo* skin samples of white Wistar rats

Optical clearing agent: aqueous 70% glycerol solution with the addition of 10% dimethyl sulfoxide (DMSO) (n=1.4450 at 589 nm)

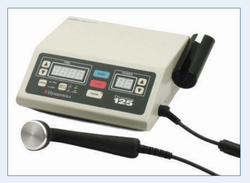
Equipment: USB4000-Vis-NIR spectrometer (Ocean Optics, USA), spectral range 450-950 nm

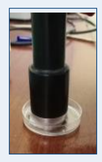
Ultrasound system Dynatron 125 US (Dynatronics, USA), frequency 1 MHz, power 1.5 W, continuous mode.



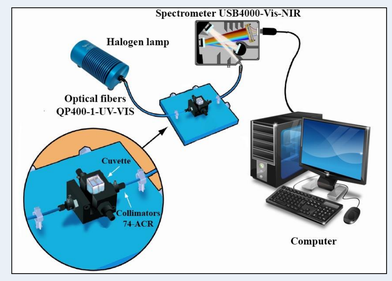


Registration of collimated transmittance spectra





3 min of US impact



Registration of collimated transmittance spectra of immersed sample

Data proccessing

Since optical clearing process includes tissue dehydration and molecule diffusion into the tissue, the evaluated diffusion coefficient shows the average rate of tissue optical clearing. Estimation of glycerol diffusion coefficient in tissue D is based on the minimization of the target function, which includes the calculated $T_c^{teor}(D,t_i)$ and experimental $T_c^{\exp}(t_i)$ values of the time-dependent collimated transmittance:

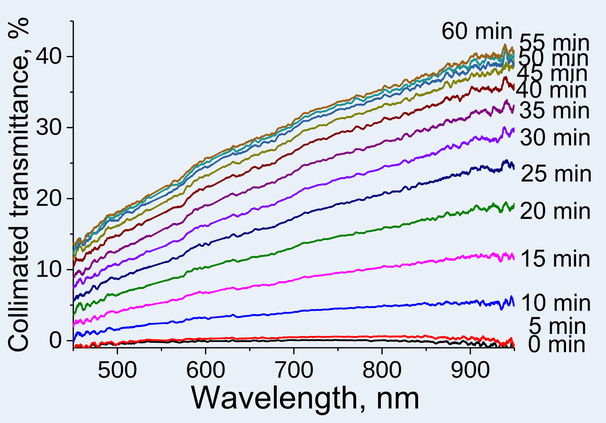
$$f(D)=\sum_{i=1}^{N_t}\left(T_c^{teor}(D,t_i)-T_c^{\exp}(t_i)\right)^2$$

Collimated transmittance is estimated as: $T_c^{teor}(\lambda,t)=exp\{-[\mu_a(\lambda)+\mu_s(\lambda,t)]\cdot l\}$, $\mu_a(\lambda)$ and $\mu_s(\lambda,t)$ are the absorption and scattering coefficients, l is the sample thickness.

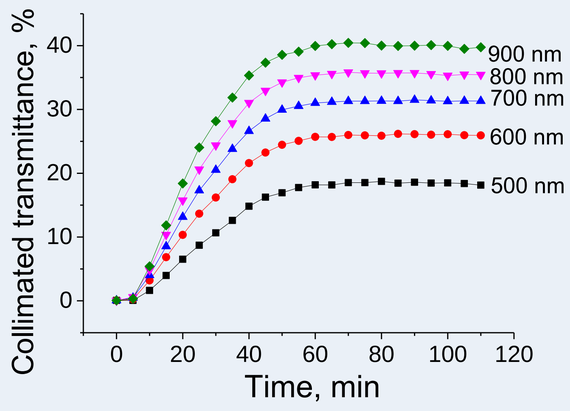
The skin permeability coefficient P for glycerol was estimated as: $P=\frac{D}{l}$.

The optical clearing efficiency of skin samples was estimated by equation: $OC_{eff}=\frac{\mu_{s\_0}-\mu_{s\_min}}{\mu_{s\_0}}$, where μ_{s_0} and μ_{s_min} are the initial and the minimal value of the scattering coefficient of the skin sample.

RESULTS



Spectra of collimated transmittance of the skin sample measured at different time at administration of aqueous 70% glycerol solution with the addition of 10% DMSO at application of US



The time dependence of collimated transmittance during optical clearing of the skin sample placed in aqueous 70% glycerol solution with the addition of 10% DMSO at application of US

Glycerol diffusion coefficient in skin (D), the permeability (P) of skin for glycerol , thickness and weight of samples before (l_0 and W_0) and after (l and W) immersion in the solution, the optical clearing efficiency of skin samples (OC_{eff}) at different wavelengths

Parameter		With US	Without US
D, cm^2/s		(2.5±1.0)×10^{-6}	(0.9±0.4)×10^{-6}
P, cm/s		(4.2±2.2) ×10^{-5}	(1.6±0.6) ×10^{-5}
l_0/l, mm		0.71±0.10 / 0.71±0.15	0.63±0.07 / 0.69±0.10
W_0/W, mg		256±48 / 194±45	259±82 / 187±76
OC_{eff}, %	500 nm	68±6	67±10
	600 nm	73±6	67±7
	700 nm	76±6	69±7
	800 nm	77±6	69±7
	900 nm	80±6	70±8

CONCLUSION

- The collimated transmittance of the skin increased with time in the studied spectral range during immersion in the aqueous 70% glycerol solution with the addition of 10% DMSO due to DMSO action on the skin, the diffusion of glycerol and tissue water, providing optical clearing of the skin.
- Higher and faster skin optical clearing was obtained at application of ultrasound to skin samples.
- The thickness of the samples did not change at application of ultrasound to skin samples.
- The weight of the samples decreased, which indicates their dehydration due to the osmotic pressure created by the agent.
- These data can be used in a wide area of biology and medicine to enhance tissue optical clearing in order for diagnostic or treatment disease by optical methods.

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