The effects of penetration and dose on *in vivo* imaging

In vivo görüntülemede penetrasyon ve dozun etkisi

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Animal models are the main subjects that were searched for the both morphological and functional changes in order to overcome the mechanism of the diseases. One of the methods to detect the anatomical changes is pathological analysis. This *in vitro* follow-up includes excision and usually sacrification of the test subjects. It requires long time- periods and many numbers of animals to distinguish a difference between the groups.

Most recently new imaging systems appear especially in the experimental research area. *In vivo* molecular imaging allows non-invasive measurement of biological processes within a living organism. These molecular systems are named as fluorescence, luminescence and radioisotope imaging techniques, which could be used within the living subjects. These *in vivo* imaging systems are able to detect the biochemical and anatomical changes without sacrification of the animal (1). This allows to follow-up same pathology or physiology in the same animal during a certain time-period. These types of devices are also suitable for behavioral studies, calcium imaging, cancer and nervous research. Some models are able to make measurements without requiring anesthesia, so that problems caused by anesthesia are eliminated and reduce the time for animal preparation. This does not only save the excess usage of animals and money but also save time (1). Imaging systems have recently taken their place in *in vivo* imaging of small animal models. These systems allow high-resolution, multi wavelength fluorescence, luminescence and radioisotope imaging in small animal models. They also include the radiographic X-ray imaging screen (1). Some models provide 3D/2D image. These systems have their novel line of organic fluorescent nanoparticles for *in vivo* imaging. These dyes (sight agents) offer brightness and sensitivity with different absorption and emission lengths. Once attached to a molecule and injected into an organism, they travel to the target biomolecules (such as stem cell) and highlight the region of interest.

Cell therapy is an important area of research activity that promises the future treatment of many diseases. Although this area is in its infancy period, there are many hopeful expectations to overcome the problems. It is related with nearly all of the clinical and preclinical disciplines and is the subject of multidisciplinary science.

Our group is working on stem cell therapies in different pathologies of clinical and experimental area. We are also excited to detect the changes following the stem cell therapies in *in vivo* models. In order to start an animal model with stem cell therapy, we wish to see the effects of the X-sight dye in Kodak imaging system. The animal experiment was carried out in accordance with the European Community Council Directive of 24 November 1986 and take place in the study that was approved by Gazi University Animal Experiments Ethical Committee (G.Ü.ET-199-19780).

The purpose of this study was to investigate the effect of depth and dose imaging of marked cells. This will be based on *in vivo* imaging studies. Male Wistar rat (300 g) was used in this study. Mesenchymal stem cells were marked with the Kodak x-sight 549 dye. After anesthesia (ketamin 50mg/kg, xylazine 10mg/kg) the marked cells (500 µL) were injected. The dye's absorption and emission values were measured as 549 nm and 569 nm. We made two injections to the right arm as superficial (just subcutaneous) and deep (5 mm deep) with 100 µL volume.

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Two injections of 100 and 200 µL in volume at the same depth (2 mm) were made to right leg. The images were taken by Carestream Health FX-PRO *in vivo* imaging system (Carestream, USA) and we measured the mean and sum intensities of the signals produced (Table 1). Arm and leg measurements were performed 4 times. Besides these, there was intensity of two more points, which we showed as white and black points on the screen (Fig. 1).

We obtained the white spots of the dye of imaging on the screen, measured the intensities and compared them by Carestream Molecular Imaging (MI) Software release 5.1 (NY, USA). When we compared both the superficial 100 µL and the deep 100 µL, we obtained approximately 2.8 times higher intensity in the superficial injection. If we compared the amount of dye at the same depth, the 200 µL dye had 1.5 times higher intensity than the 100 µL dye. Kahraman et al. (2), also described the findings from the *in vivo* fluorescence imaging and concluded that it provides functional data indicating the approximate location, magnitude. Boschi et al. (3), described the results as resolution was always better than 1.3 mm when the source depth was less than 10 mm.

Table 1. Measurements of intensities from the arm and the leg of the rat

Anatomical point	Mean intensity	Sum of the intensities
Arm; injected superficially with 100 µL	2298	5273211
Arm; injected deeply with 100 µL	821	1884442
Leg; injected normal depth with 200 µL	1107	2540833
Leg; injected normal depth with 100 µL	1684	3864346
White point on the screen	253	581254
Black point on the screen	7474	$1715x10^9$

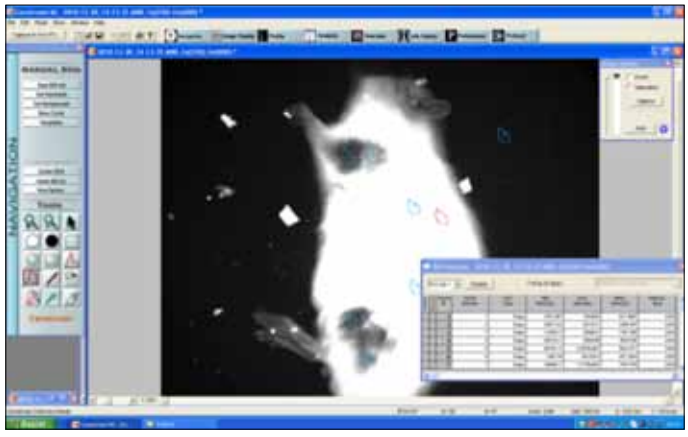

Figure 1. A 6-point of view of the density measurement of rat

It is very important to recognize the intensity of dyes to follow-up the animal models. It is useful information especially when we use dyes with the cells and to estimate the amount of cells that were recognized at the same anatomic region. The intensity getting brighter when we increase the amount of dye but it is much more important than the amount the depth of the dye that was injected.

Disclosure: Device and dye were supplied by the manufacturer (Carestream Molecular Imaging, NY, USA).

Conflict of interest: None declared.

References

1. Madero-Visbal RA, Colon JF, Hernandez IC, Limaye A, Smith J, Lee CM, et al. Bioluminescence imaging correlates with tumor progression in an orthotopic mouse model of lung cancer. Surg Oncol 2010; 27 (Epub ahead of print).
2. Kahraman S, Dirice E, Şanlıoğlu AD, Yoldaş B, Bağcı H, Erkılıç M, et al. In vivo fluorescence imaging is well-suited for the monitoring of adenovirus directed transgene expression in living organisms. Mol Imaging Biol 2010; 12: 278-85.
3. Boschi F, Spinelli AE, D'Ambrosio D, Calderan L, Marengo M, Sbarbati A. Combined optical and single photon emission imaging: preliminary results. Phys Med Biol 2009; 54: 57-62.