13th International Symposium on Particle Image Velocimetry – ISPIV 2019
Munich, Germany, July 22-24, 2019

Investigation of drug transport phenomena inside subcutaneous tissues

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Abstract

Subcutaneous injection with syringe pump and automatic pump is widely used for drug treatment which need continuous and low dose rate. Even though the drug injections have been utilized in a similar manner for a long time, advances in the design of injection machine are needed to minimize the pain, variability, or skin disorder by repeated injection. To avoid these side effects, more systematic approach on the effect of injection should be investigated and hence predictability of injection should be improved. Here, we report the effects of injection conditions on tissues using X-ray imaging technique. It visualized the characteristics of the shape and spatial concentration profile of injected fluid in porcine subcutaneous tissue and muscle tissue. The depot formation was different depending on injection condition. The dynamic movements of wetting front (WF) and variation of water contents in tissues were quantitatively analyzed. Based on the experimental analysis, the permeability of solution through the tissue was estimated depending on the permeated direction, injection speed, and tissue. We believe that this study provides promising building blocks when improving the performance of drug supplying devices and when forecasting the effect of the drug through biochemical and biomedical simulation.

1 Introduction

Drug injection is a favored manner for taking drugs to get full effects of the drug very directly and quickly. Among drug injection methods, subcutaneous injection is given in the fatty layer of subcutaneous tissue just under the skin. As subcutaneous tissue has few blood vessels, injected drugs are diffused very slowly with sustained rates of absorption. Therefore, it is effective in administering growth hormone, insulin and vaccines, which need continuous and low injection rate. Some pain medications like morphine and hydromorphone and allergic medications are also injected at subcutaneous tissue. The drugs are administered by single-shot injection with syringe or by long-time injection with automatic pump installed on the skin of body. For instance, Epinephrine comes in an automated injector that is used to quickly treat severe allergic reactions. (Ewan et al. (2016)) The insulin is administered both by insulin pump and syringe. (Yuan et al. (2016)) The injection quantity is spread over a long period of time, which resembles the normal situation in which insulin is gradually release from the pancreas.

Even though the drug injections have been used for a long time, the guideline of injection of drug solution has been updated based on the clinical situation for more proper treatment overcoming the limitations. The most dominant limitation is a considerable variability of the absorption and action of

drug formulations from patient to patient, but more from injection to injection for the patient. (Thomsen et al. (2012)) It means that patients cannot be certain to experience the same effect of similar injections. For instance, in case of insulin, the coefficient of variability of insulin absorption is reported as 15 ~ 25 % for the identical patient, and as 20~45 % among patients. (Heinemann (2002)) Another limitation is discomfort and pain by repeated injection, which would make patients skip or in worst cease the treatment.[14] This might be particularly important for chronic conditions that require life-long uninterrupted treatment, such as insulin treatment for diabetic patients. The insulin pen and pump have been improved for the convenience of the patient, but still the patients should deal with the fear of injection pain and needle-phobia. (Hamilton (1995)) In addition, frequent subcutaneous injection would lead to skin fracture, such as lipohypertrophy and lipoatrophy or adipocytes size decreased near the injection spot, respectively.

To avoid these non-expected effects of frequent injection, challenges in the design of injection devices are required based on an exact effect of dose on tissue to minimize the variability and pain during injection or to reduce the number of injections the patients need to take. Therefore, it is important to completely understand the factors of injection that influence the formation and diffusion of the drug in subcutaneous tissue. In previous studies, the effects injection conditions, such as injection volume, viscosity, needle length, and flow injection rate on depot formation and pain were researched. However, it is still controversial that which injection condition can minimize the pain and variability. Therefore, more systematic approach on the effect of injection condition should be investigated to discriminate factors relative to variability over time and hence improve predictability of injection.

In previous studies, the formation of drug depots had been visualized by histological study or X-ray computed tomography (CT), which are time consuming and the sample should be post-treated by sectioned or cryo-freezed. Many studies, such as radioactive labeling, ultasound measurement, and 3D μCT were conducted to visualize the insulin injection. However, these methods have limitations in terms of non-invasive measurements, spatial and temporal resolution to investigate behaviors of flow characteristics in subcutaneous tissue. Therefore, time resolved non-invasive measurements are essential to measure temporal variations of insulin solution related with the diffusion phenomena in subcutaneous tissue.

To overcome these limitations, we investigated the effects of conditions of the drug injection on the subcutaneous tissues in real time (Fig. 1c). 2D X-ray imaging technique was employed to investigate the characteristics of the shape and spatial concentration profile of the injected fluid. This imaging technique provides 2D images of water thickness with a short time interval. The dynamic movements of wetting front (WF) and variation of water contents in tissues were demonstrated as a function of injection time. The injections were performed in porcine subcutaneous tissue and muscle tissue. The porcine tissues were selected as a suitable model because the subcutaneous tissue layers have similar structures and mechanical properties with human tissue. The test methods were designed to replicate one-shot injection by syringe pump and long-term injection by automatic pump. The results of this study are an important knowledge when improving the performance of drug injection devices and when forecasting the effect of the drug through biochemical and biomedical simulation. Moreover, the medical X-ray imaging system has strong potential in research fields of drug injection.

2 Material and method

2.1 X-ray imaging

Figure 1 shows the schematic diagram of the experimental setup used in this study. The medical X-ray source (Varian A272) was applied in this study. The X-ray images were acquired by using A CCD camera (Hamamatsu, C9300, Japan). The X-ray beam was converted visible light in scintillator with fiber optics plate attached in front of the CCD camera. The CCD camera and X-ray tube were synchronized by utilizing a delay generator. The field of view was 36.0 *mm* × 24.0 *mm* and the

corresponding effective pixel size was 9 μm × 9 μm. The exposure time of the CCD camera was 1 second. The distance between sample to camera and X-ray generator to camera were 60 cm and 100 cm, respectively.

2.2 Sample preparation

Muscle and ssubcutaneous tissues were collected from pig and corresponding tissues were cut 4 *cm* × 4 *cm* with 4 *cm* thickness. The injected solutions in tissues were systematically visualized by using X-ray imaging technique. 75 % insulin solution which was adjusted to 100 *units/mL* (=3.6 *mg/mL*) and 25 % iodine contrast agent (Ultravist®, Bayer HealthCare Pharmaceuticals Inc.). A syringe pump (PHD 2000, Harvard Apparatus, USA) was used to input the solutions at various flow rates. 23 G needles were utilized in this study and insulin solution was injected from the tip of needle as a point source. The tip of the needle was inserted at 1cm under the surface of the tissues.

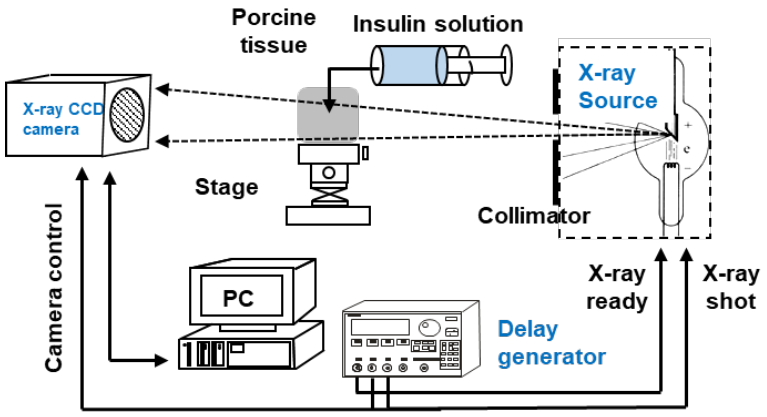

Figure 1: Experimental set-up used in this study.

3 Result and discussion

Analogous to the drug injection through automatic pump, the effect of slow infusion rate on perfusion was analyzed. The total injected solution volume was tuned as 500 μL, and the solution bolus was administered at volume infusion rates of 25 $\mu L/min$ and 100 $\mu L/min$. The injected solution into the tissue was visualized using medical X-ray facility. Once the solution starts to infuse into the tissue, solution preferentially passes by along the intercellular area between the tissues which have typical alignment. According to the alignment of tissue, depot formation of solution is different.

The temporal variations of the wetting front through the tissue are analyzed. The WF information is divided into two regions by considering injection time. In the injection region, the injection pressure is major factor compared to other forces such as capillary and gravity. In the IR, the solution permeation can be explained with Darcy's law. In the diffusion region, in which the solution is diffused by concentration gradient, WF does not dramatically increase irrespective of the injection rate.

At the initial stage of IR, the propagation velocities of vertical direction and horizontal direction are similar in both cases. On the other hand, as the last stage of IR, the solution propagates in horizontal direction faster than vertical direction. To quantitatively analyze, the aspect ratio of depot is defined

as WF_v / WF_h, where WF_v is WF in vertical direction and WF_h is WF in horizontal direction. In IR, depot aspect ratios are 0.89 ± 0.1 and 0.94 ± 0.05 at 25 $\mu L/min$ and 100 $\mu L/min$ injection rates, respectively. As the aspect ratios are almost near 1, the depot can be assumed to be spherical shape. In DR, the corresponding values are 0.85 ± 0.01 and 0.73 ± 0.02 when infused at100 $\mu L/min$ and 25 $\mu L/min$ injection rates, respectively. It implies that the solution diffuses in horizontal direction faster than vertical direction. Because of the typical tissue alignment, the solution preferentially diffuse through the wider intercellular area. The subcutaneous tissue seems to have intercellular area in horizontal direction which induces faster solution diffusion.

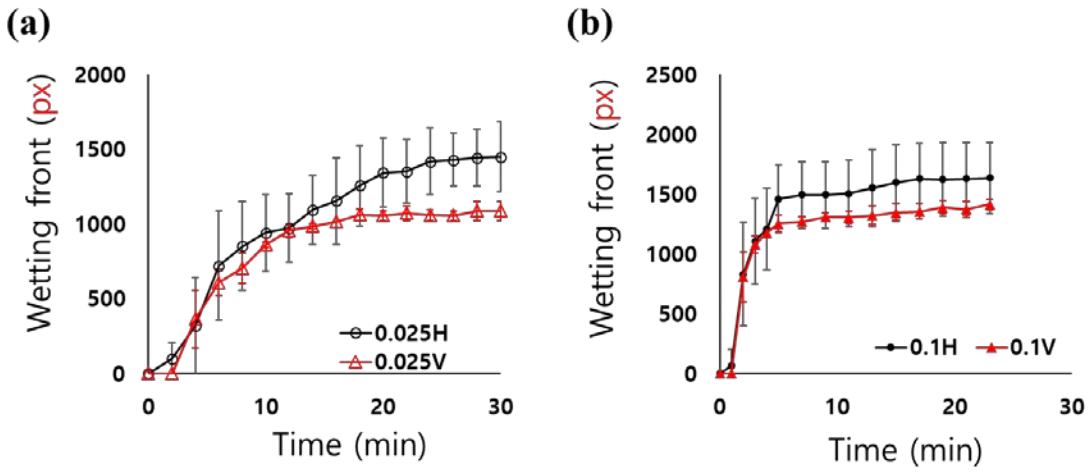

Figure 2: Effect of injection speed on the drug solution spreading. Temporal variation of wetting frong in the tissue when the drug was supplied at volume injection rate of **(a)** 25 uL/min and **(b)** 100 uL/min

Analogous to one-shot injection of drug, the effects of injection location on drug delivery in tissue were researched. The solution was injected into muscle tissues and subcutaneous tissues, and the RCS and WF inside each tissue were quantitatively compared. 500 μL insulin solution was injected into target tissues.

In case of subcutaneous injection, the solution permeates through the gap inside the tissue which has mesh network. As time goes on, the intensity of the solution is getting blur, which indicates that the solution gradually diffuses into adjacent cell. On the other hand, in case of muscular injection, the solution preferentially permeates along the horizontally aligned muscle tissue. The solution also was diffused into adjacent cells as time passed.

The horizontal WF and vertical WF are plotted according to injection location in Fig. 3a and b. Right after the injection, the horizontal WFs were more than twice larger than vertical WFs in both muscle and subcutaneous tissue. As the muscle is more permeable than subcutaneous tissue, the initial horizontal WF in muscle tissue is wider than that of the subcutaneous tissue when the identical injection force is applied. As time goes on, the value of WF approaches to quasi-saturation (QS) value, and the QS time was 2 min and 4 min in muscle and subcutaneous tissue, respectively.

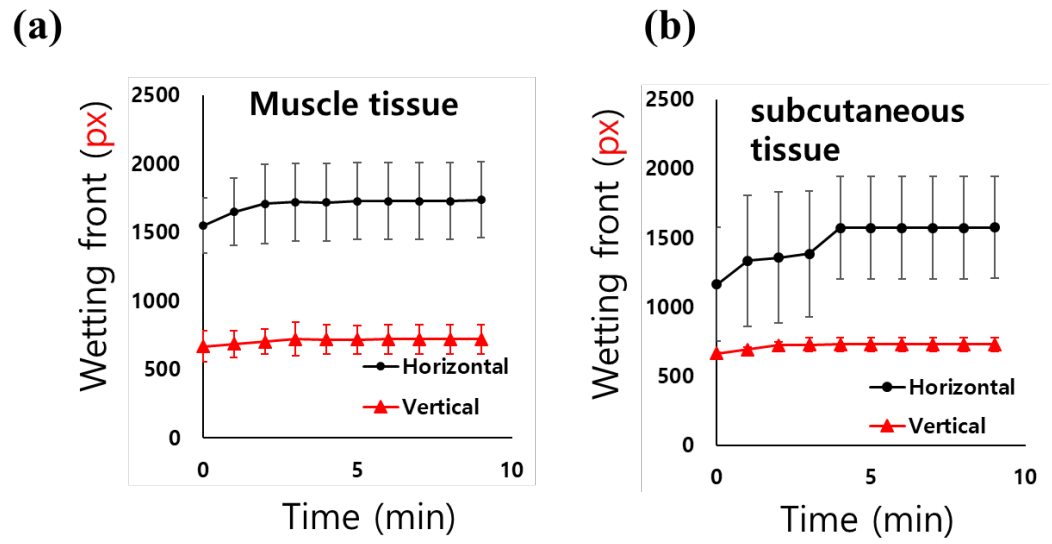

Figure 3 : Temporal variations of wetting front in the **(a)** muscle tissue and **(b)** subcutaneous tissue

The aspect ratio of the solution in subcutaneous tissue was larger than that of muscle tissue. This implies that the drug solution permeate in horizontal direction more in muscle tissue than that permeate in subcutaneous tissue. The aspect ratios in fast injection dramatically decreased compared with slow injection where the solution permeates in horizontal and vertical direction in a similar degree. This indicates that the solution permeates in horizontal direction more than vertical direction in fast injection case. This would be explained with fracture toughness of the tissue.

4 Conclusion

In this study, temporal variations of injected insulin solution inside the tissue were visualized with high spatial resolution and quantitatively analyzed according to injection speed and variety of tissues using 2D dynamic X-ray imaging technique. Injection speed is an important parameter in depot forming, because it is highly related with changing of mechanical properties in tissues. The diffusion patterns and characteristic depending on tissues were also demonstrated by single injection. With utilizing the medical X-ray facility, systematic approach on the effect of injection condition in tissues during drug infusion could be investigated. The induced results could be an important information in designing drug injection devices for clinical treatment. In technical point of view, medical X-ray facility has strong potential to investigate permeation and diffusion phenomena in *in vivo* animal disease model for clinical applications.

Acknowledgements

This work was supported by the Soonchunhyang University Research Fund

References

Ewan P, Brathwaite N, Leech S, Luyt D, Powell R, Till S, Nasser S, and Clark A (2016) BSACI guideline: prescribing an adrenaline auto-injector. *Clinical & Experimental Allergy* 46:1258-1280

Yuan J, Chen Y, Xuan Y, Cao L, Zhu J, Wang F, Zhou X, Ye Q, Liao L, Zheng Y, Zhou Q, Chen X, Chen M, and Zhou W (2016) Can the upper inner side of the thigh become a new option for insulin injection? *Current medical research and opinion*, 1-6.

Thomsen M, Poulsen M, Bech M, Velroyen A, Herzen J, Beckmann F, Feidenhans'l R, Pfeiffer F (2012) Visualization of subcutaneous insulin injections by x-ray computed tomography. *Physics in medicine and biology* 57:7191.

Heinemann, L (2002) Variability of insulin absorption and insulin action. *Diabetes technology & therapeutics* 4:673-682.

Hamilton J G, (1995) Needle phobia: a neglected diagnosis. *Journal of Family Practice* 41:169-176.