Monitoring of Intra-Tumoral Drug Pharmacokinetics *In Vivo* With Implantable Sensors

Image Credit: Olga Kononenko

By Andy Tay

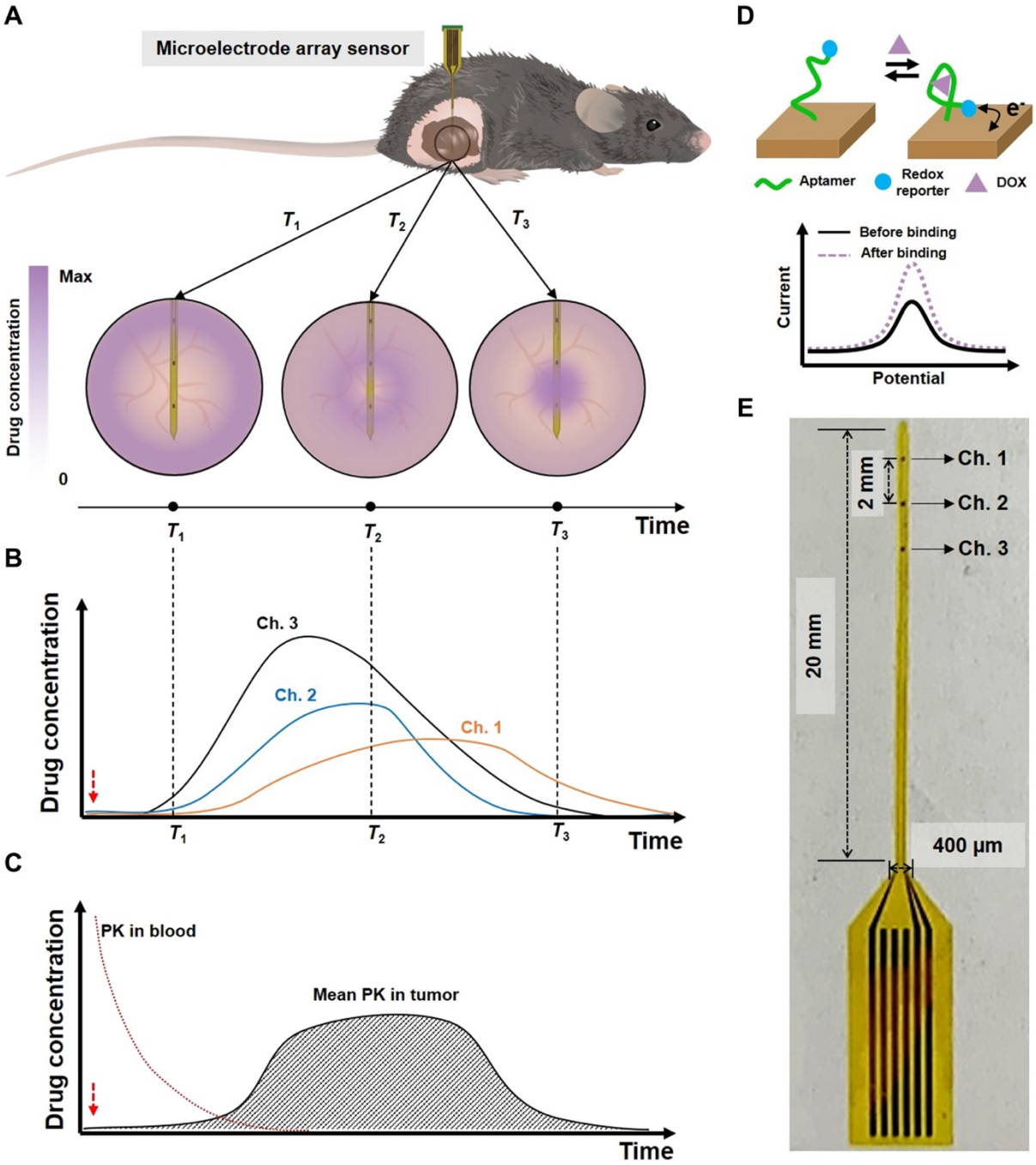
Drug discovery is an expensive process and only ~10% of all drugs in Phase 1 clinical trial gain regulatory approval by the US Food and Drug Administration (FDA), with oncology drugs having the lowest likelihood of success. The majority (39%) of those that failed to get approval were due to a poor understanding of pharmacokinetics which has an impact on a drug compound's efficacy, safety and toxicity due to under- and over-dosing of drugs.

Typically, pharmacokinetics data are derived from blood measurements of circulating drug concentrations, but this method does not capture drug absorption within tumors. This is because the tumor microenvironment is very different from healthy tissue. The former has complex vascular permeability, heterogeneous interstitial fluid pressure and disorganized lymphatic drainage which collectively impair diffusion of drugs into tumor tissues and even drug distribution in the same tumor tissue. Therefore, blood plasma pharmacokinetics data is not a good reflection of intra-tumoral drug levels. While needle-based biopsies may be used to collect tissue specimens from patients to collect tumor-specific pharmacokinetics data, they are usually performed in one tumor spot as it is not feasible or safe to perform multiple biopsies due to risk of tumor seeding.

In a recent publication authored by Professor Hyongsok Tom Soh and Professor Eric Appel and led by Dr. Ji Won Seo and Dr. Kaiyu Fu from the Department of Electrical Engineering and Department of Materials Science and Engineering at Stanford University, the team developed an electrochemical aptamer-based biosensor for real-time, multisite drug monitoring within tumor tissues in live animals (Seo et al., 2022). Using advanced nanofabrication methods, the team created a sensor that was robust, flexible, and resistant to biofouling while offering high signal to noise ratio.

“Currently, the only way to obtain tumor-specific pharmacokinetics measurements is through tissue specimens collected via needle-based biopsies. However, it is difficult to extrapolate overall drug penetration in the heterogeneous tumor tissue environment from a single sampling site, and this in turn leads to inaccurate PK assessment,’ says Seo.

“In this work, we have developed an implantable microelectrode array sensor that can collect tissue-based pharmacokinetic data by simultaneously measuring intratumoral pharmacokinetics from multiple sites. The employed nanoporous microelectrodes maintain robust sensor performance even after repeated tissue implantation and extended exposure to the tumor microenvironment. As a result, we can achieve collection of *in situ* tumor-specific pharmacokinetics data that account for tissue heterogeneity within a single animal, which is the first demonstration of continuous and multi-site measurement of a drug within a tumor in a live animal.”



Optimizing sensor *in vivo* biocompatibility and performance

The sensor is made up of an array of gold nanoporous microelectrodes integrated into a flexible polymer probe for easy implantation into tumor tissue in a live mouse (**Fig. 1**). There are also multiple microelectrodes to collect data on drug concentrations in different tumor regions. The team decided to use nanoporous structured electrodes as they can improve electrochemical detection sensitivity relative to planar electrodes due to reduced charge screening effects which they reported earlier (Fu et al., 2021). This is particularly important as *in vivo* experiments typically have high background noise, so a higher signal to noise ratio is desirable. The team also used polyimide polymer as their probe material. This is in contrast to stiff silicon or metal-based wires which can cause tissue damage, leading to inaccurate measurements.

The concept for detection is straightforward. The microelectrodes are functionalized with aptamers tagged with methylene blue redox reporter. In the presence of the target i.e., doxorubicin, a chemotherapeutic drug, the aptamer undergoes a conformal change, increasing electron transfer between methylene blue redox reporter and the electrode surface. This causes a rise in current which can be measured and correlated to target drug concentration. Importantly, as the aptamer can reversibly bind and release its drug target, the sensor can continually collect pharmacokinetics data.

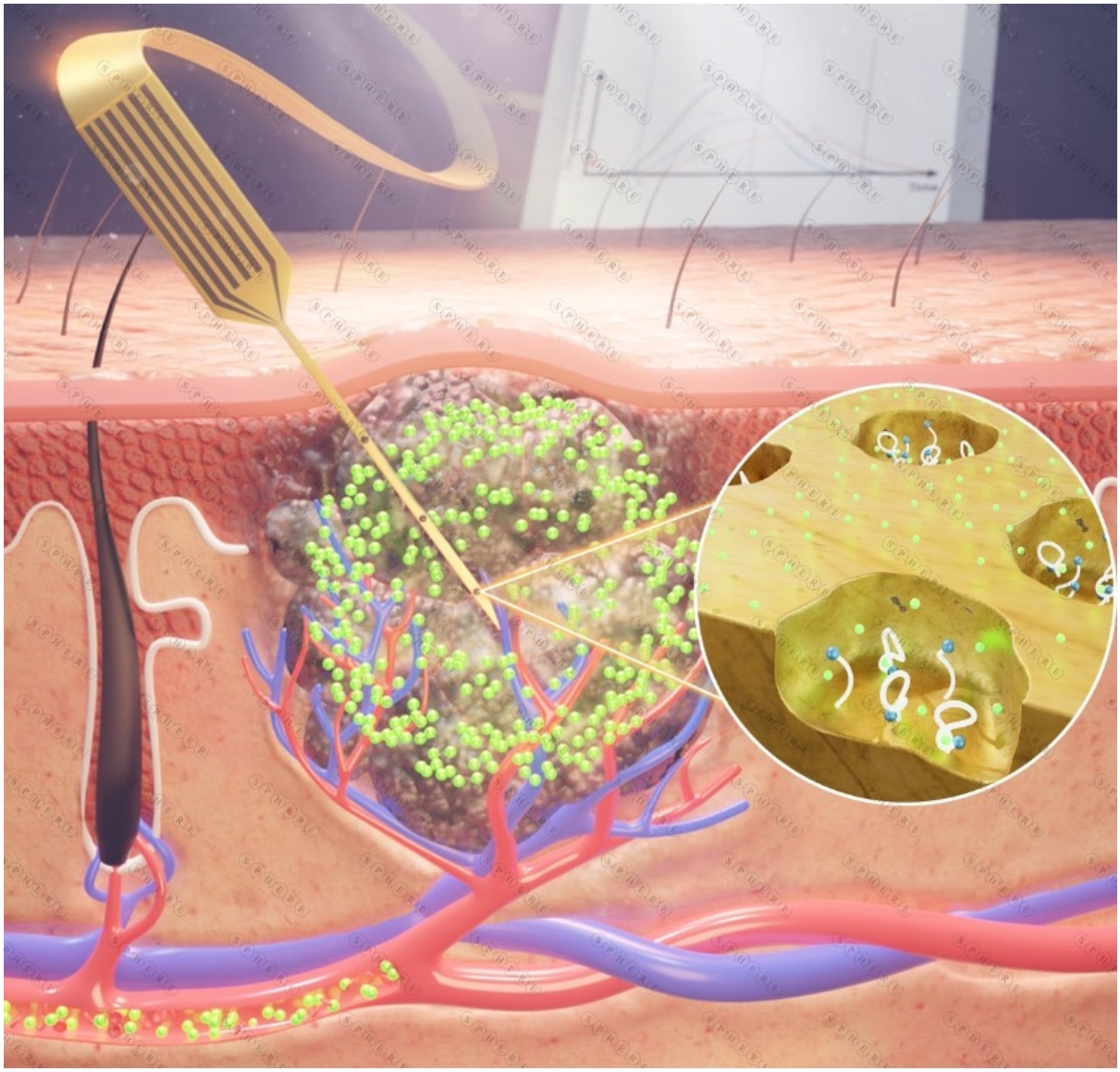


Fig. 1 Schematic illustrating the insertion of implantable nanoporous electrodes into tumor tissues for long-term monitoring of intra-tumoral drug concentration at multiple tumor regions. Credit: Soh Lab at Stanford U.

In vitro and *ex vivo* testing

The team first made use of *in vitro* testing to confirm that each of their microelectrodes has comparable sensitivity by adding doxorubicin into a buffer solution and then immersing the sensor into it. The signal gains also correlated well to various concentrations of the drug. To assess the biofouling of their sensor, the team then immersed their device in a flowing solution containing fetal bovine serum to mimic blood plasma. It was found that the nanoporous microelectrodes maintained 70% of baseline signal after 16 hours under the flowing serum, suggesting stable performance. The authors attributed this to sequestration of aptamers within nanopores that can help minimize the effects of biofouling.

Next, the authors inserted their sensor into *ex vivo* tumor tissues to understand whether there could be mechanical damage during tissue insertion and how that would affect the signals collected. The nanoporous microelectrodes were able to retain 95.1% of their baseline signal after 100 cycles of insertion and withdrawals from tumor tissues. This shows that the sensor is compatible for use in tissue as it causes minimal damage to sensor surface and functionalized aptamers.

To demonstrate that the sensor was able to measure spatially different drug concentration, the team inserted their sensor into a piece of tumor tissue *ex vivo* and injected doxorubicin into the tissues. All three microelectrode channels responded to the drug and were able to provide distinctively different signals based on their proximity to the drug injection site. The sensor was also able to provide stable detection in response to repeated drug injections and washing away of drugs.

In vivo monitoring of drug levels

The authors finally tested their device using a mouse tumor model, with channel 3 of their sensor closest to the surface and channel 1 deepest within the tumor tissue. The team found that channel 1 had the lowest signal as the center of the tumor had dense microvasculature and high interstitial fluid pressure which resulted in a lower rate of drug penetration into the tumor. Feeding their collected data into a two-compartment model, the team was able to derive a model to explain the drug concentration variability. For instance, the maximum drug concentration for channel 1 (5 mm depth within tumor) was four times lower than that for channel 3 (1 mm depth), and this was supported by their fitted model. This result highlights the difficulty of measuring pharmacodynamics data based on conventional biopsies and the value of implantable sensors.

"Our biosensor platform could offer a simple and robust tool for obtaining more physiologically relevant insights into drug PK and understanding the *in vivo* behavior of experimental drugs, thereby guiding dose selection for first-in-human studies that maximize likelihood of efficacy while minimizing dose-related toxicity. Furthermore, this platform could prove valuable for preclinical in vivo characterization of cancer therapeutics and may offer a foundation for future clinical applications," says Seo.

Promise of implantables for _in vivo_ drug monitoring

The biosensor described here can be a useful tool for preclinical analysis of pharmacokinetics data of experimental drugs to guide dose selection in clinical studies by maximizing therapeutic efficacy while minimizing dose-related toxicity. With increased channel numbers in the sensor, it can also be applied to larger animal and tumor tissues. The microelectrodes can also be functionalized with different aptamers to detect and monitor multiple drugs for potential applications in clinical drug and biomarker studies.

Source article

Fu, K., Seo, J.-W., Kesler, V., Maganzini, N., Wilson, B. D., Eisenstein, M., Murmann, B., & Soh, H. T. (2021). Accelerated Electron Transfer in Nanostructured Electrodes Improves the Sensitivity of Electrochemical Biosensors. *Advanced Science*, *8*(23). https://doi.org/10.1002/advs.202102495

Seo, J.-W., Fu, K., Correa, S., Eisenstein, M., Appel, E. A., & Soh, H. T. (2022). Real-time monitoring of drug pharmacokinetics within tumor tissue in live animals. In *Sci. Adv* (Vol. 8). DOI: 10.1126/sciadv.abk2901

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