Short Communication

Next Stage of Alternative Approaches to Animals Testing

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ABSTRACT

The use of animals in various fields including biomedical research, product safety testing, and education has increased for the development of medical technology, and advancement of science knowledges. According to the EU and US government statistics (http://www.usda.gov/, http://eur-lex.europa.eu), over 100 million animals, including mice, rats, frogs, dogs, cats, rabbits, hamsters, guinea pigs, monkeys, fish, and birds are used for research each year, of which 80% of animals were used for drug developments and toxicity test to evaluate novel drugs.

Keywords: Alternatives, Animal testing, Tissue engineering, 3D culture model, Human-on-a-chip

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In this regard, the animal testing establishments on worldwide promoted the three Rs such as guiding principles which encourage researchers to reduce the animals used in experiments, refine the pain and distress to which animals are exposed, and replace the use of animals with non-animal alternatives when possible [1]. Also, various laws and acts have been passed to bring the control over the unethical use of animals and minimize the pain to animals [2]. In that regard various alternative routes, including *in-silico* prediction based on the chemical toxicity database, *in vitro* analysis using target cell culture, and alternative organisms such as prokaryotes, protists and lower vertebrate, have been suggested to minimize or avoid the animal use in researches [3]. Compared with animal models, alternative routes present the time efficiency, less man-power and cost efficiency. However, it also presents some limitations for specific testing purposes. Actually, the single- and repeated-dose systemic toxicity, toxicokinetic, reproductive toxicity, and carcinogenicity tests are not yet replaced by non-animal method, because these researches have to need the analysis of targeted organs and overall health of the animal body. Also, computational prediction method depends on an existing database, which cannot predict the novel chemical and biomaterials. Therefore, alternative approaches must overcome these limitations to completely replace the animal testing in research [4].

Over the past 20 years, tissue engineering evolved from the field of biomaterials development and is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological functions [5]. Tissue engineering constructs have several advantages over cell-based alternatives currently in bone grafting, muscle model and cardiac models. Unlike cells in suspension or monolayers, tissue engineering constructs have a 3D-tissue formation by biocompatible scaffolds. Consequently, there are cell-cell and cell-matrix interaction within the construct that affects the cell behavior and fate in animal organ and tissue [6]. Therefore, tissue engineering technology has replaced the *ex-vivo* analysis based on organ or tissue culture derived from animals [7]. Aforementioned advantages of tissue engineering technology overcome the limitation of cell-based alternative approaches, which depicts *in vivo* microenvironment. When this technology is used with human stem cells, which have great potential in alternatives because that can realize the various artificial organs on *in vitro* condition. Although the tissue engineering technology provided the vast advancement in alternatives to animal testing related to the supply the raw

materials such as each artificial organ, this technique does not provide the opportunity to analyze the interrelated overall processes of the body occurring in the central nervous system, endocrine system, and immune system [8]. The animal and human have the extremely complex circulatory system to carry the administered drugs to different organs by blood, which changed the physiology of all organs in body [9]. Therefore, to completely replace the animal testing, the model systems must require the circulatory system to evaluate the overall response about the toxicity and efficacy of drugs and chemicals.

Huh et al. has reported the novel way of alternatives to animal testing [10]. This group has created a living lung-, heart- and gut-on-a-chip integrating microfluidic technology, which has human cells on the top, a membrane in the middle, and blood capillary cell beneath [11]. They have shown a reconstitution of the smallest possible functional unit of an organ in a microenvironment similar to that of the human body. These tissue engineering models on a chip technology could be potentially used for appropriate experiments and may replace animal models to assess and predict whether a candidate drug might be toxic or efficacious. Furthermore, this study was aimed at mimicking the multi-organs of human normal and pathological physiology within a chip as "human-on-a-chip" towards building a multi-channel 3D microfluidic system [12]. The successful development of human-on-a-chip will be able to mimic the systemic circulation of human and animal in *in vitro* condition, it will provide us the vast benefit to alternative animal testing approaches, drug development and toxicology experiments. However, human-on-a-chip remained many hurdles such as platform design and need of a common medium for different cell type [13]. Human-on-a-chip required different cell types following the target organs, thus this system will obviously require the different mediums among the cell types of well-differentiation and behavior. Oleaga et al. and Huh et al. have overcome these hurdles [14,15]. Huh et al. has shown that successful integration of ten different organs-on-chips are perfused with a universal blood substitute as well as physiologically relevant vascular connections and blood composition with arterial and venous system in humans. Oleaga et al. developed the pumpless platform system, where circulation was achieved through gravity driven flow, and chip based functional human model to evaluate multi-organ toxicity in a 4 organ system in a common defined medium was reported at first. It is evident that development of human-on-a-chip technology is a significant advancement to bridge the great gap between modern alternative route and animal testing.

Despite many efforts of developing the alternatives of the animal model system, various laws and acts for reducing the use of animals, used animals in experiments was increased during the past decade. Because modern alternative approaches are insufficient to replace an animal model, the drug developments and toxicity testing still require the animal data. To overcome this problem, many scientists were focused on 3D-culture technology and chip based approaches. The results of these novel approaches have shown the great potentials of the replacement of animal models, both in terms of reliability of results and of costs. In addition, this field will offer the simple platform of *in vitro* disease models that enable a better understanding of etiology and faster development of treatment strategies. Many studies have already shown the possibility of *in vitro* diseases model using 3D-culture and chip based approaches such as ZIKV exposure model [16], pulmonary airway model [17], and heart disease model [18]. In conclusion, the novel alternative strategies have already demonstrated the great potential for offering faster, cheaper and real time analysis of physiological response and enable to completely replace the animal testing. Now, the alternatives technology of animal testing moves on the next stage.

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