AI Augmented 3D Bio-printed High-performance Invitro Disease Model of Oral Submucous Fibrosis CELLINK Partnership Conference 2023

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INTRODUCTION

There are about 200 million brave pre cancer fighters waiting desperately for sustainable treatment solutions. The malignant transformation rate (rate of conversion to cancer) ranges from 7 to 30% in South East Asia. As most of the patients are Pan/gutka chewers belonging to the age group of 11yrs- 50yrs [1]. Despite being a potent malignant condition of such a magnitude, development of disease models and therapeutics pertaining to OSMF lack the much-needed attention. Hence, devising precise OSMF disease models is the need of the hour of the therapeutic sector to prevent it from transforming into oral cancer. Certain attributes to the absence of a reliable in vivo/invitro disease models of OSMF are lack of convenient route of administration of etiological preparation for disease induction, monitoring exposure time and dosage, mapping of OSMF phenotype [2]. Hence, the primary aim of the project is to develop highly efficient, cost effective and personalized disease model to better understand pathophysiology of OSMF for the therapeutics sector.

MATERIALS AND METHODS

In order to fabricate the disease model, a database of different histopathological grades of OSMF (fours grades I, II, III) was created. Biopsies from 30 OSMF patients and 10 normal (control group) selected randomly were collected. The tissues were fixed in 10% phosphate-buffered formalin followed by conventional processing and staining with hematoxylin and eosin (H and E) and Van Gieson stains for collagen quantification, fibroblast content, vascularity status and inception of underlying cancerous condition. Digital Data repository was created. This database served as an input for devising **customized bioink (patent application in progress)** [3]. These bioink corresponded to the intended grade of the disease to be developed. After biological, mechanical and rheological characterization of the bioinks, the OSMF disease platform was 3D bioprinted. Preparation of areca nut extracts/pan masala/pure arecoline solution: 0.2 g of powdered areca nut was dissolved 6 ml of distilled water followed by centrifugation at 15000 rpm for 30 min. A volume of 0.2 ml of areca nut solution supernatant was collected and injected into the Bio printed OSMF analogue to induce disease. The disease stage and progression were validated with digital bio twin created.

RESULTS AND DISCUSSION

The maximum width of fibrotic bands obtained at the 16th week measured 400.02 µm in the image analyzer. The other significant tissue changes were abnormally low epithelium thickness recorded at 6th week (31.25 µm). The average epithelium thickness was lower (75.49 µm) compared to the normal group, showing the most atrophic epithelium. The keratin thickness and vascularity followed an irregular trend with minimal thickness of keratin noted at the 16th week (21.37 µm). The maximum vascularity was noted in the 8th week and the minimal in the 16th week. The initial increase in the keratin thickness is probably as a part of the tissue's protective response to the irritating effects of the areca nut solutions. However, the decreased thickness in the later weeks can be explained by the continuous deleterious effects of the solutions on the tissues leading to increased damage to the epithelium and in turn a thinner keratin layer. The irregular trend followed by all the groups during the subsequent weeks may be due to the individual responses eliciting genetic diversity to the irritating stimulus. This study highlights the need to personalized medicine and how bio-printed disease models can help achieve this.

CONCLUSIONS

The technology of Bio-printing has made it possible to device animal free disease models that closer to the disease microenvironment of humans on one hand and allowed genetic and environmental inclusivity on the other. It has also set a momentum for developing disease models for rare disease which otherwise difficult to be induced in animals due to various issues.

ETHICAL STATEMENTS

The study protocol was approved by Institutional Animal Ethics Committee.

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