THE EFFECTS OF AGE and SMOKING ON ALVEOLAR BONE DERIVED STEM CELL (aBMSC) ISOLATION AND GROWTH

Celia Sommer, Chen Cao, Alex Dahlkemper, Darnell Kaigler

SCHOOL OF DENTISTRY
UNIVERSITY OF MICHIGAN

Introduction

Alveolar bone serves as a potent source of aBMSCs, which can be used in cell therapies for the regeneration of craniofacial bone defects. Developing stem cell therapies into clinical application requires standardized techniques for isolating and growing aBMSCs. The goal of this project was to determine how systemic variables such as age and smoking affect the isolation and growth potential of aBMSCs.

Method

In this pilot study, a total of 58 alveolar bone samples were collected (0.2 cc - 1.0 cc) using a bone scraper during surgical procedures requiring bone removal from systemically healthy individuals between the ages of 20 and 75 years old. The sample collection included 21 current smokers. From these samples, aBMSC populations were isolated and characterized through cell-surface marker expression and growth kinetics. Multivariate linear regression analysis was performed.

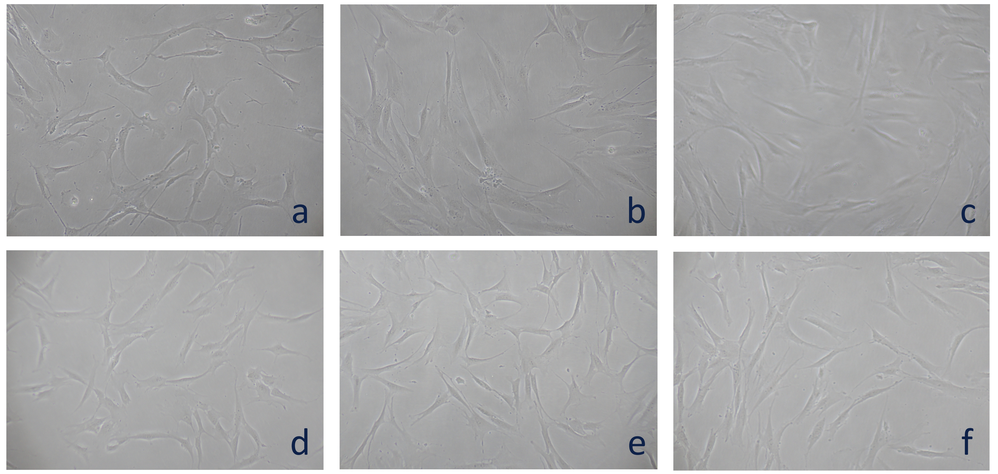

Figure 1. Photomicrograph of aBMSCs in culture at the third passage with similar confluence. Morphology is similar across all ages and smoking status. (a) Non-smoking 18-35 year old donor. (b) Non-smoking 36-55 year old donor. (c) Non-smoking 56-75 year old donor. (d) Smoking 18-35 year old donor. (e) Smoking 36-55 year old donor. (f) Smoking 56-75 year old donor.

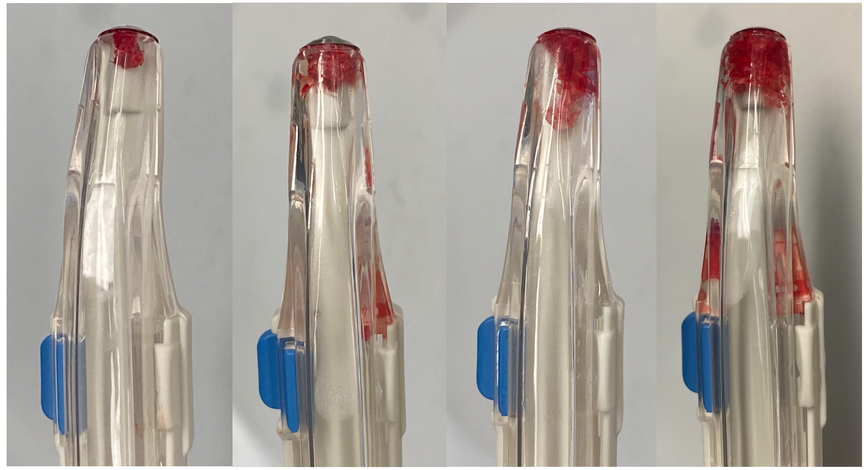

Figure 2. Photograph of alveolar bone samples collected using a bone scraper. Ranging in weight from 0.02 grams - 0.21 grams.

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Results

The ability to predictably isolate aBMSCs decreased with age among smokers. In alveolar bone samples from the smoking group, there was a 100% success rate in isolating aBMSCs from samples of younger patients compared to 50% success rate in isolating aBMSCs from older patients. Among non- smokers, the donor age did not significantly affect the ability to isolate aBMSCs. Regardless of smoking status, cell surface marker expression was consistent among all age groups with > 99% of cells expressing CD73, CD90 and CD105. Finally, while there existed pre-culture differences in the cell compositions of the samples, after 72 hours in culture these differences were not apparent in that regardless of smoking status or age, aBMSC populations had a fast proliferation rate (mean population doubling time =1.36 days, SD=0.46 days) and a high stem-cell specific cell fraction of 19-27%.

Conclusion

In non-smokers, age did not affect the ability to isolate or expand aBMSCs nor did it have an impact on their growth kinetics in culture. However, in smokers the combined effects of smoking and age had a slightly negative effect on the ability to isolate aBMSCs and their growth kinetics in culture. Further studies evaluating the osteogenic potential and regeneration capacity of these cell populations are needed to continue to translate these findings toward clinical application for the regeneration of bone defects.

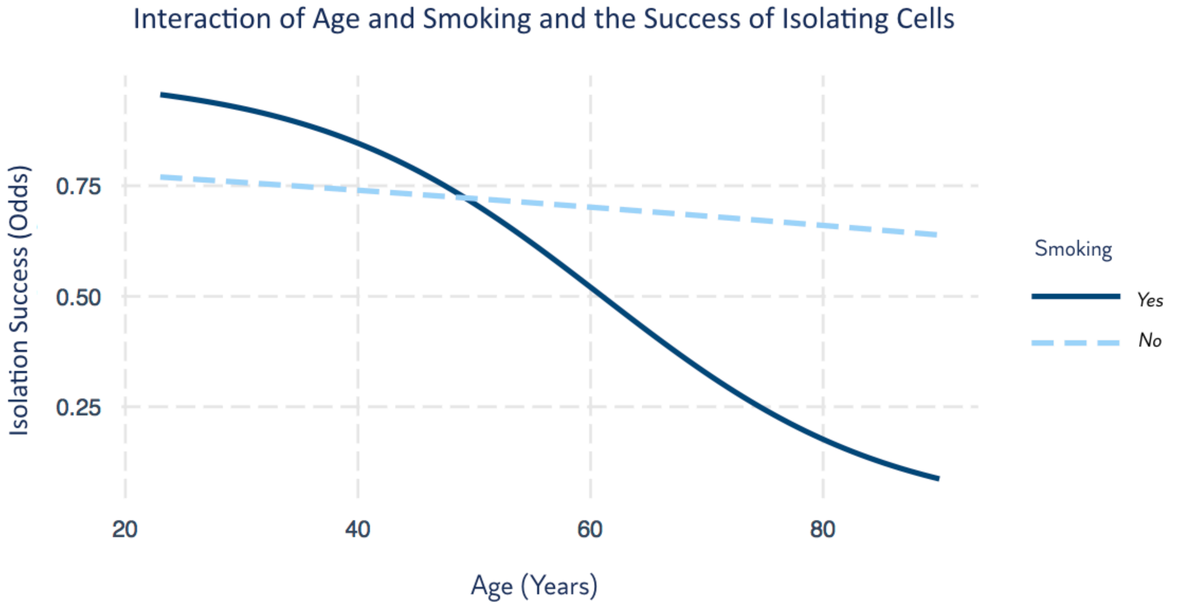

Figure 3. Interaction plot of the logistic regression between age and smoking on the capacity to successfully isolate aBMSCs. Negative correlation between smoking and age. The odds of being able to predictably isolate aBMSCs decreases among smokers with age. The odds of isolating aBMSCs remains relatively constant with age among non-smokers.