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OP20-5 Molecular and cellular aspects of smoking on fracture healing: A comparison of two models

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Objective: The detrimental effect of smoking on the fracture healing process is well documented clinically. However, the effect of smoking on the fracture microenvironment is ill defined. Mesenchymal stem cells (MSCs) play the key role in fracture healing. The aim is, therefore, to study the effects of smoking at the cellular level by comparing ex vivo MSCs (smoker *versus* non-smoker) with cells in an in vitro model (smoke-treated cells *versus* untreated cells).

Methods: Fracture haematomas were collected during operative fixation from non-smoking (n=11) and smoking (n=6) patients who sustained tibial fracture. The haematoma was explanted and maintained in culture (37°C humidified 0·05 CO_2) incubator. Cells were characterised via immunofluorescence and immunophenotyping using known MSC-antibody markers (CD29, CD44, CD105 and CD166). *Ex-vivo model:* MSCs were counted after 5 days using flow cytometer; proliferation rates were compared between non-smoker and smokers. *In-vitro model:* Cigarette smoke extract (CSE) was prepared (according to Bernhard, 2004). Cells from non-smoking patients were divided into two groups and cultured as before. One group was treated with the CSE, which was equilibrated to 20 cigarettes per day smoker and the other left untreated as control. Cell populations were counted and compared after 5 days.

Results: *Ex-vivo model:* Proliferation rates were reduced by 41·19% (SEM± 7·78%) in those cells extracted from smokers' MSCs. *In-vitro model:* Cell counting showed a reduction in the rate of proliferation of cells in the smoke treated group by 40·8% (SEM± 1·9%).

Conclusion: Fracture haematoma MSCs, when exposed to tobacco smoke; proliferate at similar decreased rate in both ex vivo and in vitro models.