Optimization of Scaffold Cell Seeding Process by Design of Experiment for Bone Tissue Engineering
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Introduction
Cell seeding into scaffolds plays a crucial role in the development of efficient bone tissue engineering constructs. Thus, the identification of key factors that quantitatively predict reproducible and efficient seeding protocols becomes imperative. In this study, optimisation of cell seeding efficiency (CSE) was investigated using design of experiment (DOE) statistical methods.¹²

Materials and Methods
A 2⁵⁻¹ factorial screening experiment (Tab.1) was set up to investigate a passive cell seeding process on two types of 3D Titanium (Ti) scaffolds (Ø6mmx3mm). The CSE response outcome was quantified by dsDNA as the percentage of initially seeded cells attached to the scaffold indicated.

The ANOVA model included all the main and 2nd order interaction effects. The statistical significance level was set at P<0.05.

Table.1. DOE of 2⁵⁻¹ factorial experiment

<table>
<thead>
<tr>
<th>Factors</th>
<th>Low Level (⁻)</th>
<th>High Level (⁺)</th>
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<tbody>
<tr>
<td>A Cell-Medium volume</td>
<td>30ul (50%)</td>
<td>90ul (150%)</td>
</tr>
<tr>
<td>B Cell-Density</td>
<td>60000 /scaffold</td>
<td>120000 /scaffold</td>
</tr>
<tr>
<td>C Cell-Type</td>
<td>hPDCs (Stem Cell)</td>
<td>SaOS-2 (Cell Line)</td>
</tr>
<tr>
<td>D Environment- Incubation time</td>
<td>30min</td>
<td>4h</td>
</tr>
<tr>
<td>E Scaffold-Type</td>
<td>Regular</td>
<td>Irregular</td>
</tr>
</tbody>
</table>

Results
CSE analysis identified a number of statistically significant main factor effects (A, C, D) and interactions (CD, AE and AB) (Fig.1a). An increase in incubation time had a positive effect on CSE, while increasing cell seeding medium volume caused a significant CSE decrease. The primary cell (human periosteum derived cells (hPDCs)) versus SaOS-2 cell line had the biggest effect on the CSE.

This experiment also showed an interaction between cell type and incubation time (Fig.1b-d), in which hPDCs incubation time had a bigger beneficial effect on CSE than that of SaOS-2 cells. CSE was also greatly influenced by interactions AE and AB, in which, an increase in medium volume caused a reduction in CSE at lower cell densities compared to a minor effect at high densities. Also the irregular scaffolds were more sensitive to medium volume changes than the regular ones.

Discussion and Conclusions
The use of a standard cell line together with a regular scaffold and a high cell density may lead to a more robust seeding process. Using a DOE strategy can help to identify and optimise critical process variables to reduce variability and assist in determining which variables should be carefully controlled during GMP production to enable a consistent clinical outcome.

References

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Disclosures
The authors have nothing to disclose.