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Effects of Surface Properties of the Allografts on the Behavior of Osteogenic Cells

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BACKGROUND

Allografts are commonly used as osteoconductive scaffolds to reconstruct alveolar bone defects by accelerating new bone formation [1,2]. Due to the differences in the manufacturers’ processing techniques, commercially available allografts possess different characteristics, which can influence the adhesion and growth of osteogenic cells and subsequent bone regeneration [1,2].

Objectives: The aim of this study was to investigate the impact of processing techniques on the adhesion and osteo-differentiation of mesenchymal stem cells (MSCs) on the allografts in vitro. To achieve this aim, we compared the osteoconductive potential of allografts manufactured by two different processes: i) the proprietary Tutoplas® solvent dehydroxylation process; and ii) conventional freeze-drying process.

MATERIALS & METHODS

Allografts: Four commercially available mineralized cancellous bone allografts including Purus® (Zimmer Biomet), Creos® (Nobel Biocare), OxalRAFT® (LifeNet Health), and Minora® (BioHorizons) were included. The chemical solvent dehydroxylation (SD) process was employed for one of the allografts, while three other allografts were prepared with the freeze-drying process (F1-D3).

Table: Distribution and Preservation Method

<table>
<thead>
<tr>
<th>Allograft</th>
<th>Manufacturer</th>
<th>Preservation Method</th>
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<tbody>
<tr>
<td>Purus®</td>
<td>Zimmer Biomet</td>
<td>Solvent dehydroxylation (SD)</td>
</tr>
<tr>
<td>Creos®</td>
<td>Nobel Biocare</td>
<td>Freeze-drying (F1)</td>
</tr>
<tr>
<td>OxalRAFT®</td>
<td>LifeNet Health</td>
<td>Freeze-drying (F2)</td>
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<tr>
<td>Minora®</td>
<td>BioHorizons</td>
<td>Freeze-drying (F3)</td>
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Brunauer-Emmet-Teller (BET) surface area analysis: The specific surface area was measured using a BET gas adsorption analyzer based on Krypton absorption.

Cell adhesion and proliferation on allografts: Human MSCs were seeded on the allografts, and cell adhesion and proliferation were then assessed at days 1, 3, and 7 using a Cell Counting Kit (CCK-8) according to the manufacturer’s protocol (Dojindo Molecular Technologies). In addition, attached MSCs were stained using a LIVE/DEAD® Viability/Cytotoxicity Kit (ThermoFisher Scientific), and the stained cells were observed using a fluorescence microscope (Olympus).

Scanning electron microscopy (SEM): Cells on different allografts were fixed in 2% paraformaldehyde for 10 minutes followed by immersion in 4% paraformaldehyde for 20 minutes. The fixed cells were then dehydrated using a graded series of ethanol (50%, 75%, 95%, 100% diluted in DI-H2O) for 3 minutes followed by a graded series of hexamethyldisilazane (HMDS; 25%, 50%, 75%, 100% diluted in ethanol) for 15 minutes. Dehydrated samples were left in 100% HMDS overnight until they were completely dry, and were imaged using a SEM (JEOL model JSM 6460LV).

Alkaline phosphatase (ALP) activity: Cells on different allografts were trypsinized and harvested at day 21, and the levels of ALP activity were quantified using an Alkaline Phosphatase Assay Kit, according to the manufacturer’s protocol (Biovision).

Statistical analysis: One-way ANOVA and Tukey multiple comparison tests were used to determine statistical differences. The p-values <0.05 were considered significant.

RESULTS

Significantly higher surface area was measured for the SD allograft compared to the FD allografts (Figure 1A; left). No significant differences in the surface area were observed between the SD allografts (Figure 1A; right). The integrity of the collagen matrix structure was preserved to a greater extent with the SD allograft as shown by the SEM micrographs (Figure 1B).

Figure 1. Brunauer-Emmet-Teller specific surface area analysis (A) and SEM images (B; x10,000 magnification) of different allografts.

Enhanced MSC adhesion and proliferation were observed on the SD allograft when compared with all the FD allografts at all time points, indicating its higher degree of osteoconductive potential (Figure 2; left). Among the FD allografts, F32 exhibited a significantly increased proliferation rate at day 7 compared with F21 and F33 (Figure 2; right). Furthermore, cells on the SD allograft appear to spread more, thereby increasing the contact area between the cells and underlying surface (Figure 3). At day 21, there was significantly increased alkaline phosphatase activity for the MSC cultured on the SD allograft, indicating enhanced MSCs osteo-differentiation on the SD compared to the FD allografts (Figure 4).

Figure 2. Cell viability and proliferation analysis of MSC on different allografts at days 1, 3 and 7.

Figure 3. Fluorescence LIVE cell staining (x100) and SEM (x1000) images of MSCs on different allografts at day 7.

Figure 4. Alkaline phosphatase activity of MSC on different allografts at day 21.

CONCLUSIONS

The proprietary Tutoplas® process preserves the natural structure of the collagen matrix and tissue integrity, which may contribute to an enhanced osteoconductive potential compared to conventional freeze-dried allografts. As a result, allografts manufactured using processes less disruptive to the collagen matrix can potentially serve as a viable alternative to autografts for bone grafting procedures.

REFERENCES