Gas Cluster Ion Beam Surface Modification of Collagen Scaffolds Enhances Cell Attachment, Proliferation and Degradation Time

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Abstract
Advanced wound healing using biodegradable scaffolds such as collagen requires rapid initial cell attachment and proliferation. However, in certain applications mechanical stability and the transition between scaffold degradation and new tissue generation is also an important factor. Currently, most commercially available collagen scaffolds use chemical and UV based treatments to increase degradation times. However, traditional chemical crosslinking produces zero-length cross links that makes the scaffolds impervious to cell infiltration rendering it less useful for applications where cell infiltration is considered desirable. Thus, there is a need to increase degradation times of bioactive scaffolds while enhancing cell infiltration and growth. Gas cluster ion beam (GCIB) irradiation is currently being evaluated for use in nano-scale modification of biological surfaces to increase cell-attachment, proliferation and differentiation. Briefly, using GCIB, a surface is bombarded with a beam of high energy cluster ions that can modify a wide variety of surface materials to a very shallow depth of approximately 10 nm without subsurface damage. We hypothesized that this nano-scale modification of biodegradable scaffolds could result in longer degradation times while enhancing cell attachment. Thus, this present study is directed to the use of GCIB processing of honeycomb collagen scaffolds to have improved cell attachment, infiltration and proliferation of cells along with increasing the degradation time of the material.

Introduction to GCIB

- Unique energetic ion bombardment technique
- Clusters are formed using Argon due to inert properties.
- Clusters that collide with surface have capability to modify material to about 100nm (GCIB) below the surface.
- Surface Treatment using GCIB and;
  - Unique energetic ion bombardment technology
  - Clusters that collide with surface have capability to modify material to about 100nm (GCIB) below the surface
- Very shallow penetration – at atomic levels
- Extremely localized process that only impacts the surface

Methods and Materials

- 3x3x2mm honeycomb collagen scaffolds, from bovine dermal Type I Achilles were GCIB irradiated to a dose of 5 x 10^13 ions/cm² or left as controls.
- Mouse MC3T3/E1 cells were seeded at 100,000 cells/scaffold in a 6µl droplet placed on an autoclaved polystyrene plate Petri dish. One collagen scaffold was placed on top of a cell suspension droplet, allowing the cells to enter the collagen scaffolds by capillary action for 2 hours at 37°C, in 5% CO2 atmosphere. After 2 hours, the collagen scaffolds were transferred to a 96 well plate and fed with 350µl of media. Media was exchanged every 3 days.
- The samples were cultured for either 1, 3 or 7 days to observe cell attachment and proliferation.
- Degradation times and total cell numbers were estimated by enzymatic digestion and quality of cell attachment was visualized using SEM imaging.

Results

Initial Findings

As seen in the graph below, the time required to completely break down GCIB irradiated collagen scaffolds was 50% longer than untreated scaffolds at Day 1, 33.3% longer at Day 3 and 50% longer at Day 7 indicating the possibility of crosslinking of the collagen fibers due to GCIB irradiation.

By SEM analysis, it was apparent that there were more cells on the GCIB-irradiated samples as compared to controls.

Hypothesis: GCIB surface modification of collagen scaffolds could enhance mechanical stability by slower degradation of the scaffold while maintaining the degree of cell attachment and proliferation, allowing for the transition to tissue.

Long term effects of GCIB treatment

A 3 month shell life study was performed in order to test the long term effects of GCIB treatment. For this experiment, honeycomb collagen scaffolds were GCIB treated with a dose of 5 x 10^13 ions/cm² or left as controls. Cell seeding was performed at three different time points in the course of the study: Day 1 (Freshly processed) 1 week and 3 months. Degradation time analysis, Histological analysis and SEM analysis were performed at each time point.

Degradation time:

Table 1: Summary of the time required to digest CTRL and GCIB treated collagen scaffolds after 1 or 7 days in culture for 3 months in culture

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time in Culture</th>
<th>CTRL</th>
<th>GCIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day</td>
<td>10 mins</td>
<td>18 mins</td>
<td>20 mins</td>
</tr>
<tr>
<td>7 Days</td>
<td>30 mins</td>
<td>35 mins</td>
<td>38 mins</td>
</tr>
<tr>
<td>1 Week</td>
<td>30 mins</td>
<td>35 mins</td>
<td>38 mins</td>
</tr>
<tr>
<td>3 Months</td>
<td>32 mins</td>
<td>38 mins</td>
<td>43 mins</td>
</tr>
</tbody>
</table>

As seen in the table above, the time to digest untreated honeycomb collagen scaffolds averaged 16 minutes after 1 day in culture and to 28 minutes after 7 days in culture. However, with GCIB treatments the time to degradation of the collagen scaffolds increased almost 1.4 fold after 1 and 7 days in culture.

SEM and Histological analysis:

- SEM analysis:

  The cells isolated from enzymatic digestion of the collagen scaffolds were quantified using an automated cell counter in order to estimate the total number of cells attaching and proliferating on the untreated and GCIB treated honeycomb collagen scaffolds. Results from the cell quantification assay indicated a 50% change in the total cell numbers on GCIB treated collagen surfaces. Over time, cells start to proliferate on the surface of collagen scaffolds; however, there appears to be substantially more cells on the GCIB-irradiated samples.

- Histological analysis:

  Folding of collagen scaffolds was observed using Histological imaging. Alizarin Red staining was used to confirm osteogenic differentiation of the cells. Image magnification 5X, Image capture using Image J.

- As seen in the graph below, the time required to completely break down GCIB irradiated honeycomb collagen scaffolds averaged 16 minutes after 1 day in culture and to 28 minutes after 7 days in culture. However, with GCIB treatments the time to degradation of the collagen scaffolds increased almost 1.4 fold after 1 and 7 days in culture.

Conclusions

- GCIB irradiation results in increased degradation times of the honeycomb collagen scaffolds
- GCIB irradiation enhances total cell attachment and proliferation on collagen scaffolds
- GCIB irradiation seems to accelerate the rate of remodeling of the collagen scaffolds
- In a two dimensional environment, GCIB irradiation induces rapid osteogenic differentiation of MSCs cells.

Taken together, these results suggest that GCIB surface modification of collagen scaffolds could enhance recalcitrant wound healing, where tissue regeneration requires speed of cell attachment and proliferation as well as mechanical stability provided by slower degradation of the scaffold, allowing for the transition to tissue.