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

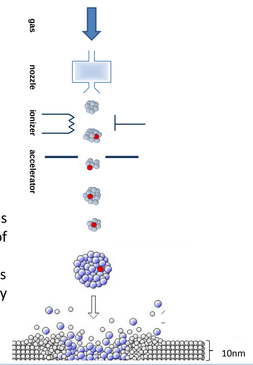
Kshama Doshi, MS; Sean R Kirkpatrick; Laurence JB Tarrant, PhD; Richard C Svrluga; Joseph Khoury, PhD
Exogenesis Corp. Billerica, MA 01821

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 biopolymer 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 by 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 biodegradation 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 10nm (GCIB) below the surface.
- Surface Treatment using GCIB and:
  - Permits changing surface properties without changing the surface chemistry of a material
  - 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 Atelocollagen were GCIB irradiated to a dose of 5 x 10^{13} ions/cm^2 or left as controls
- Mouse MC3T3/E1 cells were seeded at 100,000 cells/scaffold in a 6µl droplet placed on an autoclaved perfluoroalkoxy 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% CO_2 atmosphere. After 2 hours, the collagen scaffolds were transferred to a 96 well plate and fed with 250µl 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

Initial Findings

As seen in the graph below, the time required to completely breakdown 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.

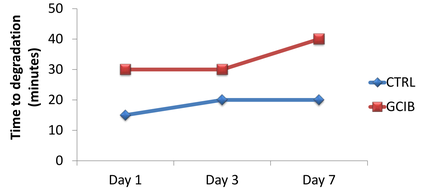


Figure 1: Time to complete digestion of untreated and GCIB treated collagen scaffolds over 1, 3 and 7 days in culture

Cell number quantification:

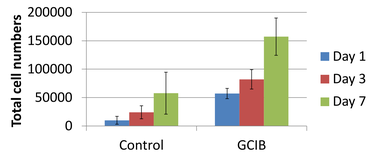


Figure 2: A graph representing the cell numbers recorded on CTRL and GCIB treated collagen scaffolds after 7 days in culture during the 3 month shelf-life study

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 that there was an increase in the total the 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.

SEM analysis:

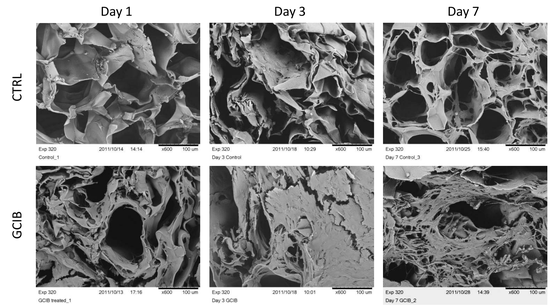


Figure 3: Representative SEM images of CTRL and GCIB treated collagen scaffolds indicating cell attachment over 1, 3 and 7 days in culture

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

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 shelf life study was performed in order to test the long term effects of GCIB treatment. For this experiment, honeycomb collagen scaffolds were irradiated with GCIB to a dose of 5 x 10^{13} ions/cm^2 or left as controls. Cell seeding was performed at three different time points in the course of the study: Day 1 (Fresh processed) 1 week and 3 months. Degradation time analysis, Histological analysis and SEM analysis were performed at each time point.

Degradation time:

Table 1: A summary of the time required to digest CTRL and GCIB treated collagen scaffolds after 1 or 7 days in culture during the 3 month shelf-life study

Treatment	Time in culture	Time after treatment			Avg digestion time (mins)
		1 Day	1 Week	3 Months	
CTRL	1 Day	10 mins	18 mins	20 mins	16 ± 5.29
	7 Days	36 mins	19 mins	29 mins	28 ± 8.5
GCIB	1 Day	27 mins	21 mins	20 mins	23 ± 3.7
	7 Days	40 mins	26 mins	55 mins	40 ± 14.5

As seen in the table above, the time to digest untreated honeycomb collagen scaffolds averaged to 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:

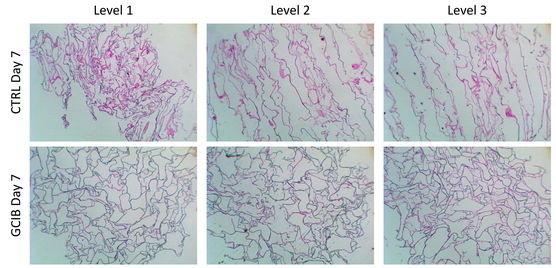


Figure 4: Representative histological images of CTRL and GCIB treated collagen scaffolds sectioned at three different levels along the depth of the scaffold. Image magnification 5X.

Histological images of the cell seeded honeycomb collagen scaffolds after 3 months of treatment and 7 days in culture revealed differences in the distribution of cell attachment and proliferation on the collagen scaffolds.

On the GCIB treated samples, cells appeared to attach throughout the scaffold in clusters. A change in the pore size of the GCIB treated scaffolds presented some evidence of matrix remodeling as well. However, the cell attachment on the untreated scaffolds was evident mostly at Level 1 and very dispersed at the other levels. SEM images of the cell seeded honeycomb collagen scaffolds after 7 days in culture over the course of 3 months were similar to Figure 3.

GCIB treatment induces rapid osteogenic differentiation

In order to test the effect of GCIB irradiation on differentiation of cells, a preliminary study was performed. In this study, human mesenchymal stem cells were seeded onto untreated or GCIB treated collagen coated coverslips and induced using osteogenic differentiation media.

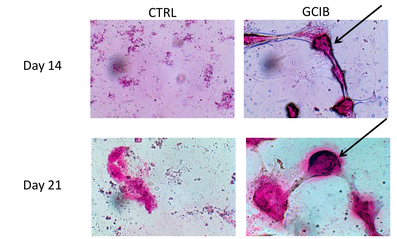


Figure 5: Representative images of Alizarin Red staining on CTRL and GCIB treated collagen coated coverslips indicating osteogenic differentiation. Image magnification 5X.

Alizarin Red staining on the collagen coated coverslips indicated a significant amount of calcium deposition on the GCIB treated coverslips as compared to the untreated surface at Day 14 and Day 21. Thus, GCIB treatment might be able to induce rapid differentiation of cells in the right environment, thus accelerating the wound healing process.

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 hMSC 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.

Questions or Further Information
Contact:

Joseph Khoury, Ph.D.
Director of Cell and Molecular Biology
Exogenesis Corp.
20 Fortune Dr
Billerica, MA 01821
Tel: (978) 439-0120
Email: jkhoury@exogenesis.us