Bone Marrow Absorption and Retention using Capillary Action via Micro-Channel Structure

Daniel S. Oh[+1], Francis Y. Lee[1], Hesham Tawfeek[1], Phillip H. Lim[1], Danaa Ganbat[2], Yoon Hyuk Kim[2]
[1]Columbia University Medical Center, New York, NY; [2]Kyung Hee University, Yongin, Korea.

Statement of Purpose: The absence of a functional environment in most grafts has hampered the potential for clinical applications and the success of bone tissue engineering [1]. To this day, successful grafting has been dependent on the loading of pre-expanded mesenchymal stem cells (MSCs) and/or large amounts of growth factors, requiring elaborate laboratory techniques and excessive costs [2]. For this reason, inducing bone formation using natural environmental cues has become a major focus in tissue-engineering research. During the past decade, scientists have employed a vast number of techniques to mimic natural bone characteristics such as pore size, porosity, interconnectivity of the pores, and permeability through synthetic grafts [3]. These factors collectively play a role in cell attachment, proliferation, and differentiation as well as in nutrient flow and cell communication, all of which are crucial for proper bone healing. Many research groups have designed scaffolds with highly organized internal structures to provide cells with a surface for attachment and the potential to gain access to nutrients. However, these highly organized structures have merely been synthetic platforms that have limited potential in overcoming the challenge of passive cell growth. Mostly, these approaches have resulted in the in vitro growth of tissues with cross-sections of less than a few μm to mm from the external surface [4]. To this end, we have proposed a scaffold with superior fluid retention capacity, which may absorb bone marrow cells and present growth factor-containing body fluids such as blood clots and/or serum. Our novel trabecular-like scaffold has a three-leveled structure composed of primary-macro-pores, secondary-micro-channels, and nano-pores. The combinatorial effects of these internal structures result in a host-adapting construct that enhances cell migration and adhesion from the host bone marrow throughout the entire scaffold.

Results: A computer aided design model indicated a significant geometrical difference depending on the presence or absence of the micro channel structure for the same unit volume. Whereas the porosity was slightly increased (8.8%), the surface area was significantly increased (41.96%) (Fig. 1 & Table 1). Indeed, an increased surface area is one of the major factors to maximize cell attachment onto ideal scaffolds. By fundamental physical nature, μm-size channels exhibit highly effective fluid absorption. The data showed highly effective absorption and retention of bone marrow throughout the entire 2 cm-height scaffolds. It showed more than 10 times faster absorption capacity than cadaveric human bone (Fig. 2). SEM reveals scaffold architecture with open pores and interconnected rod-like struts. Micro-CT images demonstrate complete interconnectivity and pore size ranging from 300-400 ㎛ and porosity of 80.15±1.28% as compared to the third human lumbar vertebra. Combinatorial effects of these internal structures result in a host-adapting construct that enhances cell migration and adhesion from the host bone marrow throughout the scaffold. With initial seeding density of 1 x 10^6 MC3T3 cells, in despite of the rigorous conditions, such as having only half of the scaffold covered by media. After 24 hours and 48 hours, mobilized cell front reached approximately 83% and 90% of the scaffold height, respectively (Fig. 3).

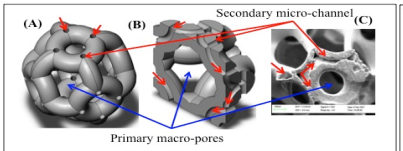

Fig. 1. Schema of trabecular bone composed of primary maro-pores and secondary micro-channels (A) and cross section view (B) by CAD. SEM image represent cross section image of engineered micro-channel along trabecular septum (C). Red arrows represent fluid flow through micro-channels to continuous supply for nutrient and waste, etc.

Table 1. The comparison of porosity and surface area.

	Porosity	Macro-pore	Trabecular beam	Micro-channel	Surface area
Non-Channel	79.5%	300 μm	100 μm	None	8.307 mm²
With-Channel	86.5%	300 μm	100 μm	50 μm	11.793 mm²
	+ 8.8%			++++	+ 41.96%

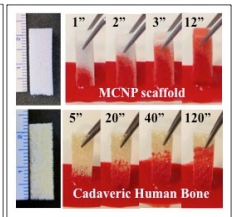

Fig. 2. Comparison of the 'Absorption Property' using bone marrow: MCNP scaffold demonstrated more than10 times faster ingress than human allograft (1x1x2.2 cm).

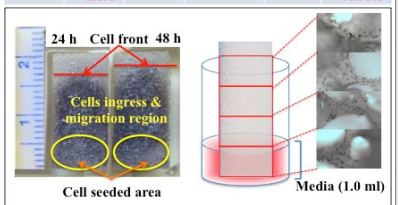

Fig. 3. Cells ingress and migration into MCNP scaffold (1 x 1 x 2 cm height) after 24 and 48 hours at cell density of 1 x 10^6 (left). The cells are seeded at bottom of the scaffold. Then the scaffold was placed into 48 well culture plate followed by 1.0 ml non-osteogenic media was added into well (right, H&E stain).

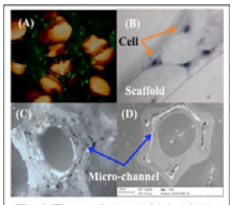

Fig. 4. The attachment and migration onto MCNP scaffold surface and micro-channel: (A) fluorescence photographs of calcein labeled 20x, (B) H&E stain showed clear cell morphology include nucleus and cytoplasm 100X, (C) infiltration and migration into micro-channel 40x, and (D) SEM.

Conclusions: A highly efficient in cell ingress and habitation, the micro-channel scaffold was successfully developed using a polyurethane template coating method to fertilize tissue engineering. The absorption and retention capability of the engineered scaffold was tested using bone marrow. The ingress and habitation of cells within the scaffold was tested using rigorous culture conditions with a 2 cm-height scaffold. Useful mechanical strength (~3.8 MPa) and high porosity (82%) were achieved on the scaffolds composed of 320 μm pore size. The cells were well relocated and proliferated on the scaffolds. The seeded cells were actively mobilized and settled down. Most importantly, we confirmed the presence of cells in the micro-channels, suggesting that the micro-channels not only enhance fluid ingress but also provide additional space for cell habitation (Fig. 4).

References: [1] Karageorgiou V. Biomaterials. 2005;26:5474-5491. [2] Arinzeh, TL. Biomaterials. 2005;26:3631-3638. [3] Goldstein AS. Biomaterials.

2000;22:1279-1288. [4] Freed LE. Adv Drug Deliver Rev. 1998;33:15-30.