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ECM analog technology: a simple tool for exploring cell-ECM dynamics

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1. ABSTRACT

Cells in a functional tissue display a highly interactive relation with their neighboring cells and associated biochemical milieu. Serious disruption in the existing homeostatic balance in the extra-cellular matrix (ECM) may lead to abnormal response by the cells. With insufficient understanding of Cell-ECM interaction and in absence of simple tools for *in vitro* cell-culture in 3D, we still have to rely on the data generated by growing cells in 2D. In order to comprehend Cell-ECM dynamics it is important to recreate *in vivo* like microenvironment in 3D. Senescence and loss of function commonly observed in cells cultured in 2D are expected to be surmounted using such tools. Unlike prevailing belief that 3D culture is required only for tissue engineering (TE) and regenerative medicine, simple and easy to handle tools for *ex vivo* 3D culture may lead to greater impact. With the potential to improve our understanding about cellular behavior, both in normal and abnormal surroundings, they may eventually influence the diagnosis. Here we discuss some of the tools for cell culture in 3D, made available through novel cell-interactive ECM analog® technology.

2. INTRODUCTION

Despite perceptive limitations and serious inadequacies experienced while growing cells in flat surfaces, researchers and pathologists are stuck with the 2D methods of cell culture. This impediment can be attributed to a great extent to the non-availability of simple and easy to handle tools for practicing 3D cell culture (1). Tissue engineering has been the prime motive behind evolving new scaffolds (2, 3), though the impact of having a versatile and handy tool for 3D culture is expected to be enormous (1). With the potential of bringing our understanding about cellular behavior to a new level, 3D cell culture systems are expected to influence present practices of both diagnosis and therapeutics. With the advent of novel, synthetic but biocompatible polymers scientists started looking into the options of growing cells on scaffolds of different shapes and sizes so that they can adopt that shape eventually. Synthetic polymers possess enough strength and are moldable into any desired shape (4). Perfect molding can facilitate creation of ear, tube/vessel and bladder like mimics. However, scaffolds from synthetic polymers lack the suppleness and the

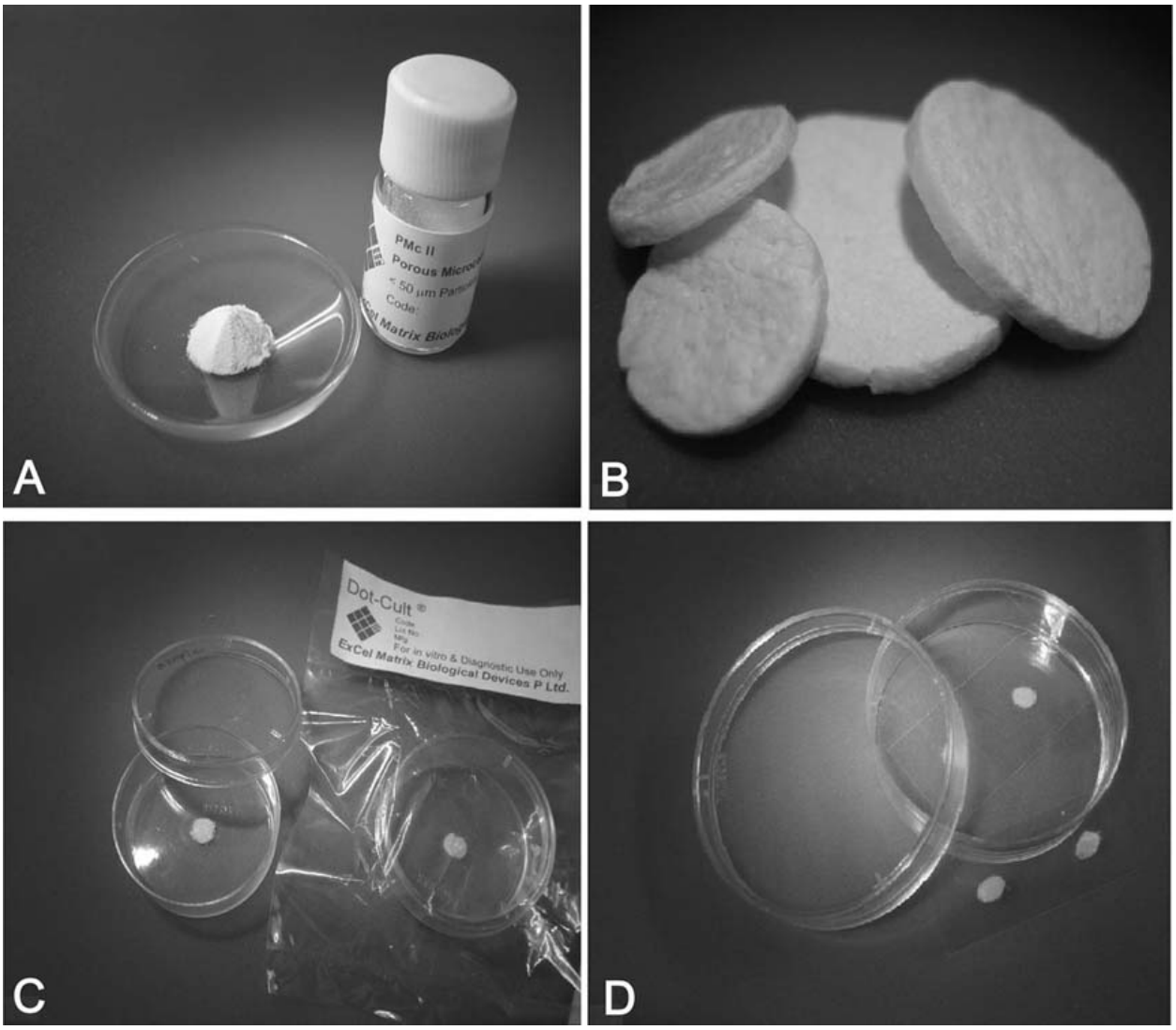

Figure 1. Available formats of ECM analog® technology A. particle or micro-bead format (PMc) for large scale vaccine production, B. disk format (ECM graft) for *in vivo* graft study, C. dot format for 3D cell culture research and D. dual dot format for confocal microscopic studies after growing cells in 3D.

conformational and spatial features of real extra-cellular matrix (ECM) which are important for cell interactivity. Thus, 3D scaffold crafted from synthetic biomaterial though offer a substitute for 3D cell-culture *in vitro* their outcome cannot be extrapolated to valid cellular response *in vivo*. Providing physical space in 3D might step up the information in comparison to what we get by culturing cells in 2D cell-culture devices (5, 6) but, in absence of cell-interactivity as presented through extra-cellular matrix (ECM) *in vivo*, the cells growing on synthetic matrix continue to remain functionally sub-optimal.

Realizing this, attempts have been made to develop scaffolds from natural polymers like dextran, silk, chitosan etc. (7). Hydrogels (8) and scaffolds derived from collagen (9) are also evaluated for optimal cell growth and differentiation. Other natural resources like tumor matrix (10) urinary bladder and de-cellularised extra cellular matrix (ECM) of adipose tissue (11, 12) too are explored as complete substitute for ECM. Scaffolds derived from non-immunogenic, natural polymers being cell-interactive are found to be better ECM mimics (13). Besides, they provide ample scope for customization for diverse applications (14). Unlike synthetic 3D scaffolds where only chemical conjugation to the backbone could facilitate customization, those from ECM derived biopolymers can be easily adapted both through chemical conjugation and/or physical adsorption.

Here we discuss the ECM analog technology which offers not only a convenient alternative to synthetic 3D scaffolds in terms of cell interactivity but is the only ECM substitute which is available in thermally stable, easy to handle and most familiar formats (Figure 1a-d). Being constituted from ECM derived collagenous macromolecules it offers a better *in vivo* mimic where

Table 1. Comparative merits of ECM-analog technology over available synthetic and naturally derived 3D-Cell culture systems.

3D-Cell culture Systems	**Constituents**	**Sterlization**	**Handling**	**Microscopy**	**ECM mimicking**	**Versatility**
Matrigel (Natural)	Tumor derived ECM	γ-radiation	Cumbersome, multi-step	Transparent	Actual tumor ECM	Limited
ECM-analog Scaffold (Artificial)	collagen-hybrid/Semi-synthetic	γ-radiation, Heat /autoclavable, Alcohol, Ethylene oxide	Simple, similar to conventional 2D-culture	Transparent	Fundamental/essential physical and biochemical ECM mimic	Unlimited (customizable to tissue specific ECM through chemical and physical methods)
Synthetic Scaffold (Artificial)	PS,PCL,PLGA &/or Calcium phosphate	γ-radiation, ethylene oxide	Simple, similar to conventional 2D-culture	Opaque	Physical ECM mimic	Limited

spatial modifications can be achieved through physical or chemical adjustments. The porous scaffold can be uniquely customized by simple impregnation or chemical incorporation of tissue specific growth factors for culturing different types of cells. It is expected that mere physical adsorption over such cell-interactive scaffolds might be sufficient for incorporating most of the tissue specific ECM cues (6).

The micro porous scaffold generated from ECM analog technology that facilitates 3D culture is cell-interactive, microscopically transparent, trypsin sensitive and above all thermally stable. It mimics the essential physical and biochemical features of natural ECM while incorporating the desired features of both the naturally derived ECM substitute like matrigel and also that of a synthetic polymer scaffold. Table 1 shows comparative merits of the novel biomaterial which has inbuilt strength to maintain the 3D framework like that of synthetic scaffolds while remaining cell-interactive.

Existing in particle form the ECM-analog scaffold is versatile enough to permit diverse permutation combinations for replicating natural micro-environment *ex vivo*. It can be autoclaved without loosing its architecture and holds reasonable shelf life. Thus, for the first time it is possible to study cell-dynamics in 3D environment with the scope of acquiring information regarding the impact of individual ECM components. It is well established that the cells exist in a dynamic relation with their immediate extracellular microenvironment. Though there are two major interactive elements of this extracellular microenvironment which influence the cells; the extracellular matrix (ECM) and the neighboring cells, however, studies indicate that the impact of cell-ECM interaction is more prominent as many a time it mediates the communication between the cells. ECM represents an integrated, usually fibrous, scaffold-matrix that provides structural and temporal signaling along with the matrix-fluid comprising of hormones, growth factors and other small signaling molecules. Cell-ECM dynamics plays an important role in facilitating and regulating various cellular processes. The complex cell-ECM interaction may lead to migration, morphogenic signaling, homeostasis and apoptosis or may even end up causing tumorogenesis or other pathological conditions (15). It is now believed that the matrix directs and influences the cellular response via definitive type of cell adhesion (16). Changes in ECM are sensed by the cells through different signaling mechanisms including receptors present on their surfaces. A signal instilled through ECM contributes immensely and directs the cells to undertake a specific pathway that may lead to migration, proliferation or differentiation. Not much is known about the factors which regulate cell-signaling. However, concentration and in certa*in situ*ations presentation through the fibrous (solid) ECM network are important for optimal signaling response (17, 18). Physiologically active cells release molecules that alter the microenvironment and surrounding cellular response, which in turn can bring change in their own response. Thus, ECM organization and cell response are interdependent and exhibit a bidirectional relation. Little is known about this dynamic communication between the cells and its ECM that apparently is the chief/focal source of deviation from the healthy, homoeostatic equilibrium under pathological circumstances. In absence of appropriate 3D-scaffolds where growth factors, hormones and other ECM molecules could be presented through solid support for *ex vivo* analysis the cell-ECM response could never be approximated reasonably. With the availability of cell-interactive 3D scaffold it is feasible now to evaluate the effect of growth factors, hormones and other signaling molecules while growing cells in ECM like ambience *in vitro*. Possibility of creating and assembling the scaffold in particle, disk and sheet format further widens its application spectrum (Figure 1a-d).

3. SCAFFOLD ARCHITECTURE

The electron microscopic study of scaffold particles generated through ECM analog technology establishes the presence of interlinked pores of different dimensions. The SEM (scanning electron microscopic) images of the dry, uncoated particles reveal inter-imbedded pores of different sizes, which become even more distinct after gold-coating (Figure 2a & b). Thus, each scaffold particle is composed of porous network where interconnected pores of varying size, largest being up to 40-42 micron (uncoated) are available for accommodating cells at different growing stages (Figure 3c & d).

These particles swell 4-5 times their volume on wetting and transform into a micro-porous hydrogel with swollen pore walls (Figure 2c & d). It is clear that the porous architecture of the particles is maintained even after soaking, which is a requisite feature for their potential use

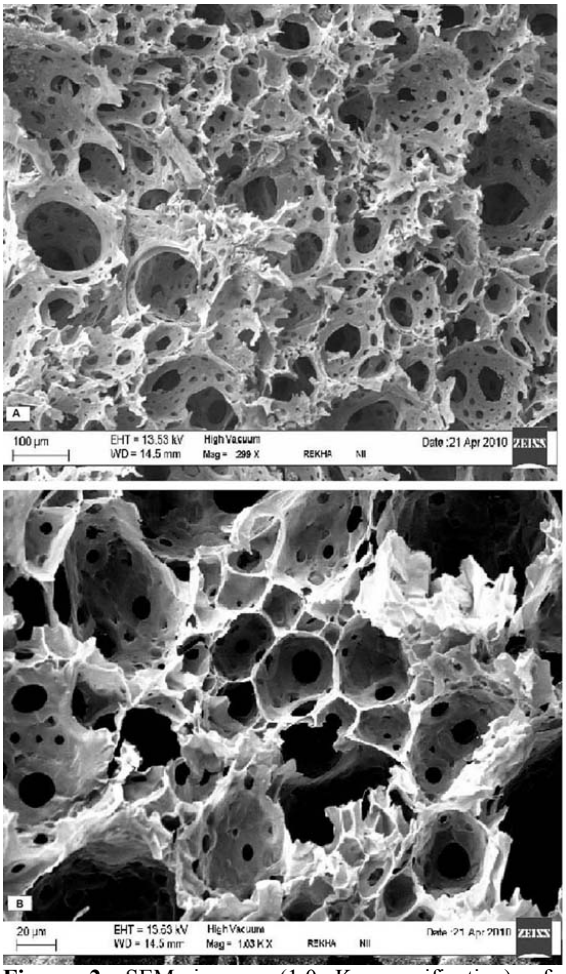

Figure 2. SEM images (1.0 K magnification) of a randomly chosen scaffold particle (ECM analog® Technology) A. uncoated, B. gold-coated, C & D. soaked in water (different views).

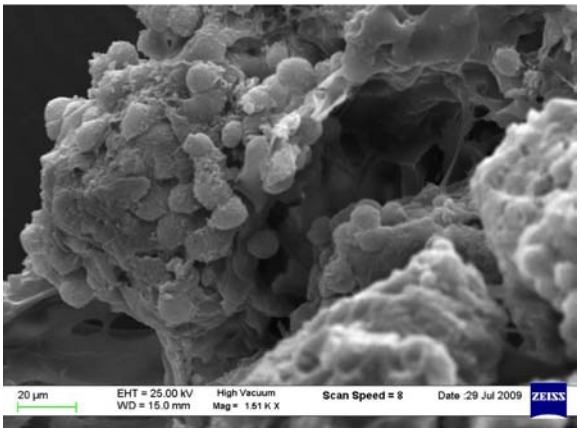

Figure 3. Size and wall measurement of the pores in a randomly chosen scaffold particle (ECM analog® Technology) on SEM images (500 x) of A. uncoated, B. gold coated C & D. particle soaked in water.

in 3D cell culture or for that matter as therapeutic carrier (19, 20). The bloated walls are adequately soft to permit the movement of newly generated cells while allowing free diffusion of nutrient medium. Bigger pores offer space for cell expansion in three dimensions while smaller ones facilitate the free flow of nutrient medium. The tenderness of biomaterial also helps in localized retention of nutrients. The interconnected pores through soft, flexible, gel like walls are expected to allow cells to grow, move and rearrange and organize themselves in a manner similar to those *in vivo*. This freedom of movement and rearrangement definitely does not exist in synthetic scaffolds. It is further observed that the porous network of the scaffold though strong enough to sustain cells in 3D, is degradable by Trypsin and Pepsin like enzymes. This proves handy for optimizing and retrieving the cells grown in 3D by selectively degrading the scaffold. The cells can be pelleted, counted or stained for desired study. Scaffold is biodegradable and when inserted in a disk format takes 6-8 weeks for complete degradation in mice. Thus, the disk format can be used conveniently for studying the behavior of an implant *in vivo*. The desired graft, for example the insulin producing recombinant cell mass can be grown/generated on the disk *ex vivo* and then inserted for systematic study of the graft's immunological and physiological response *in vivo*. It is established that human islets that lead to senescence and loss of function in monolayer can be expanded without loosing glucose responsiveness in fibrin gels which allows them to maintain their 3D architecture (21).

4. FUTURE EFFORTS

A judicious blend of synthetic and cell interactive bio-material may yield a desired spectrum of strength and cell interactivity recommended for growing different types of tissues (4). Analogous to innate ECM, the cell-interactive 3D scaffold functions as a reservoir by retaining the signaling molecules in its moist and tender walls. Such scaffolds therefore, can be used to evaluate the impact of various hormones and growth factors at different concentrations under specified experimental set up. We are trying to develop an array of semi-synthetic biomaterial through novel designs of ECM-macro-conjugates. The novel hybrid-biomaterial is intended to integrate the mechanical strength with optimal cell interactivity so that the resultant 3D scaffold can be imparted with desired suppleness. It is important to inform that physical elasticity of ECM plays an important role in guiding the differentiation of pleuripotent stem cells (22).

3D culture is the fundamental need for growing cells *ex vivo* that are functionally equivalent or closer to their counterparts *in vivo* (1). By culturing cells in ECM mimicking 3D environment we can expect better perception of normal *vs.* abnormal cells *ex vivo*. A microarray technique which helps in probing the combination of ECM macromolecule required for cell differentiation (23) can be extrapolated on ECM analog scaffold for deciphering the precise impact of various constituents on optimal cell functioning and also in providing direction to cell differentiation. Functional validation of differentiated cells would certainly be more reliable at scale higher than micro-array chip. Having a

handy tool to replicate the tissue specific microenvironment *ex vivo* is a great deal of achievement not only in the field of tissue engineering (24) and regenerative medicine but also in the course of drug discovery and delivery (6). Drug efficacy and sensitivity assays performed on 3D systems are expected to be more reliable and accurate. Thus, a quick adaptation to the new assay systems is going to help not only the diagnosis and prognosis but also abridge the path of new drug discovery where most of the failures occur due to the mismatch of *in vivo* with the *in vitro* cellular response against the drug. Thus, with the availability of an affordable, simple to use model and tools for cell culture in 3D we look forward to an informative revolution.

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6. REFERENCE

1. Ranjna Dutta, Aroop Dutta: Cell-interactive 3D-scaffold; advances and applications. *Biotech Adv* 27, 334-339 (2009)

2. R. Perez-Castillejos: Replication of the 3D architecture of tissues. *Materialstoday* 13, 32-41 (2010)

3. Lorenzo Moroni, Jennifer Elisseeff: Biomaterials engineered for integration. *Materialstoday* 11, 44-51 (2008)

4. Adam Engler, Maureen Griffin, Shamik Sen, Carsten Bönnemann, H. Lee Sweeney, Dennis Discher: Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J Cell Biol* 166, 877-887 (2004)

5. Wei Liu, Marika Bergenstock, Wing Lau, Wei Sun, Qing Liu: Comparison of osteogenic cell differentiation within 2D and 3D culture systems. 2nd World Congress of the Tissue Engineering and Regenerative Medicine International Society (TERMIS) Seoul, Korea August 31 - September 3, 2009

6. Ranjna Dutta: In search of optimal scaffold for regenerative medicine and therapeutic delivery. *Therapeutic Delivery* 2, 231-234 (2011)

7. Jungwoo Lee, Meghan J Cuddihy, Nicholas A Kotov: Three-dimensional cell culture matrices: state of the art. *Tissue Eng. Part B Rev.* 14, 61-86 *(*2008)

8. Mark W. Tibbitt, Kristi S. Anseth: Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotech Bioeng* 103, 655-663 *(*2009)

9. R Montesano, P Mouron, M Amherdt, L Orci: Collagen matrix promotes reorganization of pancreatic endocrine cell monolayers into islet-like organoids. *J Cell Biol* 97, 935-939 (1983)

10. Fridman R, Giaccone G, Kanemoto T, Martin GR, Gazdar AF, Mulsine JL: Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. *Proc Natl Acad Sci USA* 87, 6698-6702 (1990)

11. Ji Suk Choi, Hyun-Jin Yang, Beob Soo Kim, Jae Dong Kim, Jun Young Kim, Bongyoung Yoo, Kinam Park, Hee Young Lee, Yong Woo Cho: Human extracellular matrix (ECM) powders for injectable cell delivery and adipose tissue engineering. *J Cont Rel* 139, 2-7 (2009)

12. Stephen Badylak, Donald Freytes, Thomas Gilbert: Extracellular matrix as a biological scaffold material: Structure and function, *Acta Biomaterialia* 5, 1-13 (2009)

13. R Warren Sands and David J Mooney: Polymers to direct cell fate by controlling the microenvironment, *Curr Opin Biotechnol* 18, 448-453 (2007)

14. Nathan Blow: Cell culture: building a better matrix. Nature Methods, 6, 619-621 (2009)**.**

15. Ranjna Dutta, Aroop Dutta: Comprehension of ECM-cell dynamics, a prerequisite for tissue regeneration. *Biotech Adv* 28, 764-769 (2010).

16. Allison Berrier, Kenneth Yamada: Cell-matrix adhesion. J Cell Physiol 213, 565-573 (2007).

17. Richards Hynes: The extracellular matrix: not just pretty fibrils. *Science* 326, 1216-1219 (2009).

18. Francesco Ramirez, D.B. Rifkin: Cell signaling events: a view from the matrix. *Matrix Biol.* 22, 101-107 (2003).

19. Wenhui Chen; Yasuhiko Tabata; Yen Wah Tong: Fabricating tissue engineering scaffolds for simultaneous cell growth and drug delivery. *Curr Pharm Design* 16, 2388-2394 (2010)

20. Ranjna Dutta: Drug carriers in pharmaceutical design; Promises and Progress. *Curr Pharm Des* 13, 761-769 (2007)

21. Gillian M. Beattie, Anthony M.P. Montgomery, Ana D. Lopez, Ergeng Hao, Brandon Perez, Margaret L. Just, Jonathan R.T. Lakey, Marquis E. Hart and Alberto Hayek: A Novel Approach to Increase Human Islet Cell Mass While Preserving β-Cell Function. Diabetes 51, 3435-3439 (2002)

22. Adam Engler, Shamik Sen, H. Lee Sweeney, Dennis E. Discher: Matrix elasticity directs stem cell lineage specification. *Cell* 126, 677-689 (2006)

23. Christopher Flaim, Shu Chien, Sangeeta Bhatia: An extracellular matrix microarray for probing cellular differentiation, *Nature Meth* 2, 119-125 (2005)

24. Valerie Tsang, Sangeeta Bhatia: Three-dimensional tissue fabrication, *Adv Drug Del Rev* 56, 1635-1647 (2004)

Abbreviations: ECM: extra-cellular matrix, PMc: Porous microcarrier, SEM: scanning electron microscope, 3D: three dimensional

Key Words: ECM, Extra Cellular Matrix, Porous, Microcarrier, 3d, Cell Culture, 2d, Devices, Scaffold, Graft, Implant, Biopolymer, Review

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