

## **Biomimetic 3-D matrix for high-density cell culture**

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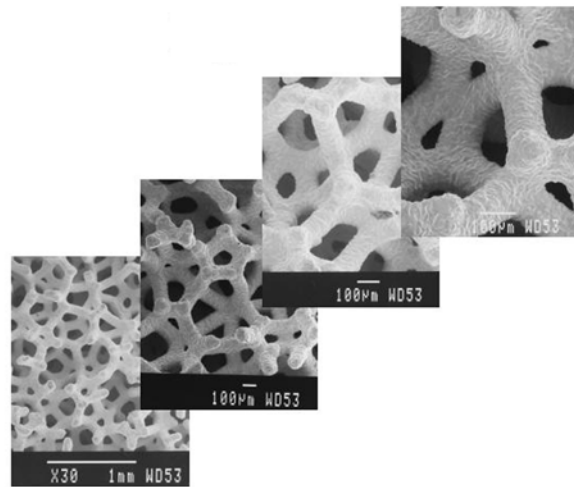
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Please note that the Spinner System and Matrix Cassettes in this published article are now referred to as the SW-Spinner System and Tuning Fork Assembly respectively.

Cell culture is a core technology for basic research, drug discovery and R&D. However, some of the fundamental biological processes currently under investigation require primary or differentiated cells that are difficult to grow in conventional cell culture systems. Other experiments require large numbers of cells that are arduous to collect in research laboratory settings.

The new Cytomatrix™ Spinner System (CSS) available from Cytomatrix, LLC Inc., Woburn, MA., supports the efficient growth of adherent cells *in vitro*. The Cytomatrix™ itself, illustrated in Figure 1, is a three-dimensional cell growth scaffold composed of niobium-coated carbon. It has a regular dodecahedral structure with continuous channels and interconnected pores that give it a porosity of 80% to 90% and a high surface area:volume ratio of 60 to 70 cm<sup>2</sup>/cm<sup>3</sup>. These structural features provide a biomimetic microenvironment that promotes the growth of adherent cells and maintains normal architecture and cell-cell associations.



**Figure 1.** Cytomatrix™ scaffold of biocompatible niobium-coated carbon shown at progressive magnifications from 30X to 100X, illustrating the rigid but completely porous structure made up of regular dodecahedrons (courtesy of Implex Corp., Allendale, N.J.).

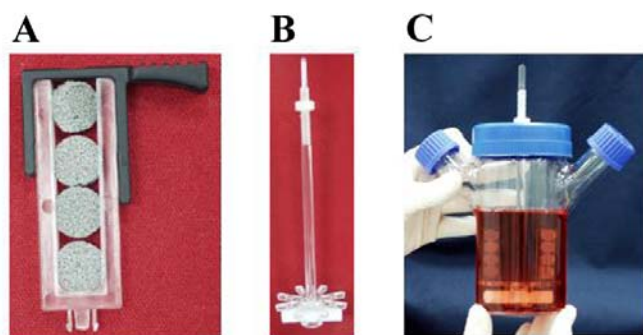
The CSS culture environment supports the growth of a wide variety of primary cells, cell lines and tumor cell lines, examples of which are listed in Table 1. All of the adherent cell types tested to date in the CSS have grown to high densities with excellent viability. Some of these cell types are notoriously difficult to grow in conventional culture, such as porcine hepatocytes, human primary bone marrow stroma and primary breast and ovarian tumor cell isolates.

**Table 1. Examples of cell types that have been cultured successfully in the Cytomatrix™ Spinner System.**

<u>Tumor cell lines</u>	<u>Established cell lines</u>
<ul style="list-style-type: none"> <li>• 3133 (human breast carcinoma)</li> <li>• JORP (human melanoma)</li> <li>• MCF-7 (human breast carcinoma)</li> <li>• SAOS-2 (human osteosarcoma)</li> </ul>	<ul style="list-style-type: none"> <li>• 293 human endothelial kidney cells (HEK)</li> <li>• 3T3 murine fibroblasts</li> <li>• 5/9 Mα 3-18 Chinese hamster ovary cells (CHO)</li> <li>• AFT024 mouse bone marrow stroma</li> <li>• C2C12 mouse myoblasts</li> </ul>
<u>Heterogeneous primary cell isolates</u> <ul style="list-style-type: none"> <li>• Human bone marrow stroma (HBMS)</li> <li>• Human skin</li> <li>• Keratinocytes</li> <li>• Melanoma skin biopsies</li> <li>• Mouse thymic stroma</li> <li>• Ovarian biopsies</li> </ul>	<ul style="list-style-type: none"> <li>• COS African green monkey kidney cells</li> <li>• HepG2 human hepatocytes</li> <li>• HI-5 insect cells</li> </ul>

## Flexible configurations

Cytomatrix units are available as disks or rectangular slats 2.5 mm thick. These units are contained in easy-to-handle cassettes that are arranged vertically around the center of a carousel-like starwheel assembly. An integrated magnetic stir bar is located at the base of the assembly,



**Figure 2. The Cytomatrix™ Spinner System.** A) Cassette Assembly: 9 mm matrix disks assembled into a cassette with black handle for handling. B) Starwheel Assembly, C) Operational 125 ml Spinner System.

as illustrated in Figure 2. The assembled unit fits into a specially modified spinner flask that is designed to be used in conjunction with a magnetic stir plate in a standard cell culture incubator. Slow rotation of the starwheel at 2 rpm facilitates even exposure of all matrix units to the same culture microenvironment through uniform convective flow.

The Cytomatrix™ Spinner System is currently available in two sizes. The 125 ml flask can accommodate up to 8 cassettes each holding four 9 mm disks. The disks are designed to fit into multi-well plates (48 well and larger). The 500 ml flask can accommodate up to 8 cassettes each holding either two 30 mm disks or one 30 x 60 mm rectangular slat. The larger flasks also can accommodate up to 16 cassettes (2 per arm of the starwheel assembly) each holding four 9 mm disks for a total of 64 Cytomatrix™ units.

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Cell culture in the CSS is flexible and scalable: total growth capacity can be adjusted simply by selecting an appropriate flask size and number and type of cassettes. The Cytomatrix™ supports very high cell densities by exposing all cells to a consistent growth environment. Most of the cell lines grown on the 9 mm disks achieve densities of 2 to 4 x 10<sup>7</sup> cells/ml, which is 10- to 50-fold higher than microcarrier systems cultured under similar conditions.

## Cell culture right out of the package

The disposable Cytomatrix™ matrix units are provided pre-packaged in cassettes, sterile and ready to use. The user simply pipettes cells directly onto the matrix units. Once inoculated seeded cassettes are placed in an incubator for 2 to 4 hr to allow the cells to attach to the Cytomatrix™ scaffold. The cassettes are then snapped onto the starwheel assembly and placed in a spinner flask containing enough medium to completely submerge the seeded units.

Most cell types grow well both at low densities immediately after seeding and at higher densities as they cover the matrix surface and infiltrate the matrix pores. There is no need to split and passage cultures through step-wise expansion at low dilutions. This simplifies culture maintenance procedures and reduces the risk of contamination. Cells can be harvested from the matrix units at any time either by enzymatic digestion with trypsin or Accumax™ (Innovative

Cell Technologies, La Jolla, CA), or by non-enzymatic removal such as cell dissociation via EDTA.

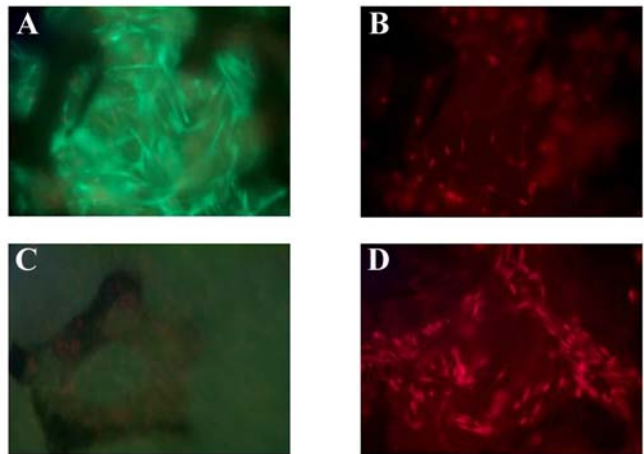
### ***In situ* visualization**

Cells can be examined *in situ* directly on Cytomatrix™ units. The extent of cell growth in the matrix can be assessed by macroscopic examination of matrix units that have been treated with a protein stain such as crystal violet. The matrix units are malleable enough that they can be sectioned with a razor blade, or other more sophisticated histology tools and examined by bright-field, fluorescent, electron or confocal microscopy. The viability of cells growing in the interior of the matrix can be viewed by simultaneous live/dead discrimination with fluorescent stains, as shown in Figure 3. Similarly, cells that have been engineered to express fluorescent molecules such as green fluorescence protein (GFP) can be visualized directly by fluorescent microscopy.

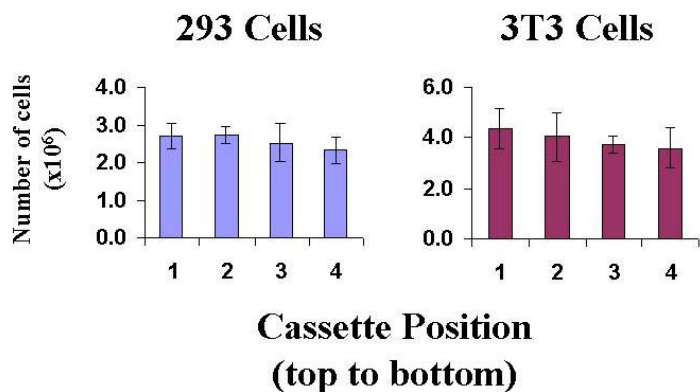
### **Aliquot sampling**

One unique advantage of the Cytomatrix™ Spinner System is its capacity for “aliquot sampling,” which is the aseptic removal of individual matrix units from a flask without disrupting the remaining units within the culture. The regular geometry and physical characteristics of the Cytomatrix™ ensures even exposure of cells to the culture microenvironment so that all of the matrix units in a single culture have comparable growth kinetics, cell yields, viability, and functional characteristics such as protein secretion. As a result, the individual matrix units in each flask represent highly uniform replicates (Figure 4).

For example, serial samples for time-course experiments can be collected quickly and efficiently by removing the required number of matrix units with forceps under aseptic conditions. The spinner flask then can be returned to the incubator for further culture and subsequent sampling. Similarly, multiple matrix units can be used for experimental manipulations that must be carried out in replicate. This eliminates the extra steps of harvesting, counting and aliquoting cells from conventional cell culture, and reduces experimental variability that can result from counting errors and recovery losses. This is important as cell-laden matrices can be used directly in cell-based assays with dramatically increased consistency between and among the matrix units. This translates to a decrease in test-to-test variability in cell-based assays.



**Figure 3. *In-situ* live-dead discrimination of primary human bone marrow stroma cells on the Cytomatrix™.** A) Interior section of Cytomatrix after 12 weeks in culture, stained with the vital stain calcein and visualized by fluorescence microscopy; all cells fluoresce green, indicating that they are viable. B) Same field as in A but visualized for the supravital stain ethidium bromide D-1; few cells fluoresce red, indicating nearly all are viable. C) Cells killed via incubation in 70% ethanol prior to staining with vital stain calcein. Notice the absence of positive cells demonstrating the specificity of the live cell stain. D) Same field as in C except visualization was to detect dead cells. Notice all of the cells stain positive demonstrating the ability to detect the presence of dead cells via this assay.



**Figure 4. Consistent aliquot sampling of cell lines grown in Cytomatrix™ Spinner System.** 3T3 mouse fibroblasts and 293 HEK cells were seeded at  $2.5 \times 10^5$  per 9 mm disk, cultured to subconfluence in 125 ml spinner flasks for 5 days (3T3 cells) or 7 days (293 cells) and harvested with Accumax. Each cell line was grown separately in spinner flasks containing 2 matrix cassettes with 4 disks each, and the position of each disk in the matrix cassette was noted when the cells were harvested. Viable cells were counted manually after trypan blue staining. Mean  $\pm$  SD. Each bar represents 6 replicates. Similar results were obtained with 5/9 M $\alpha$  3-18 cells and MCF-7 cells (data not shown).

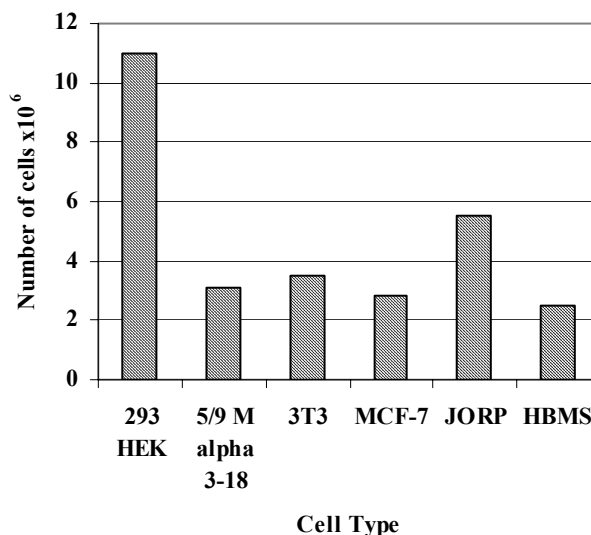
### Cryopreservation directly on Cytomatrix units

Cryopreservation is an important method for ensuring long-term access to specific cell populations, and Cytomatrix can be used to expedite the process of freezing and reconstituting cell cultures. After culturing, cells can be frozen directly on Cytomatrix™ units in cryopreservation medium and stored in cryovials. After they are thawed, they may be rinsed into fresh medium and placed directly in culture in multi-well plates. Alternatively, conventionally frozen cell pellets may be thawed by standard methods and then seeded directly onto Cytomatrix™ units for growth and expansion, obviating the need to expand cells by serial passage in plastic. Freshly thawed

cells that are seeded directly onto matrix units have growth rates and viabilities comparable to logarithmically growing cells seeded directly from plastic culture.

### Bench-scale biomass production.

For experiments that require large numbers of cells, the Cytomatrix™ Spinner System provides a convenient system for bench-scale production of bulk biomass. Due to the increase in surface area, each fully-grown 9 mm disk can hold several million cultured cells when grown to confluence, depending on the cell type. For example, up to 11 million 293 HEK cells can grow on a single disk. The maximum capacities of 9 mm disks for several different cell types are shown in Figure 5. Therefore, a single 125 ml culture flask that is fully loaded with 32 disks can produce from 60 to 350 million cells, depending on the cell type. This is roughly equivalent to 5 to 7 conventional



**Figure 5. Cytomatrix™ cell growth capacity** Maximum cell content of individual 9 mm matrix units for different cell types grown to confluence in the CSS. Refer to Table 1 for cell nomenclature.

T75 flasks. Importantly scale up from small 9 mm disks to large 30 mm disks and larger is linear.

### **Heterogeneous cell cultures**

The Cytomatrix™ Spinner System is ideal for cell types that are difficult to grow by conventional methods. For example, it can be used to maintain heterogeneous primary cell cultures such as melanoma tumor isolates that are recovered from very small skin biopsy samples. Primary tumor cells collected by needle aspiration have been grown successfully on matrix units starting at seeding densities as low as 10,000 cells per 9 mm matrix unit.

Human bone marrow stroma cells, the small fraction of adherent cells present in mononuclear cell preparations of whole bone marrow, are difficult to maintain by conventional methods, in part because stromal cells tend to peel off plastic culture dishes after several weeks in culture. However, they can be selectively captured on the Cytomatrix™ and cultured for several months with excellent viability. These units can then be used in cell-based assays such as cytokine secretion in co-culture studies with exogenously added cells such as tumor cells.

### **Conclusion**

The Cytomatrix™ is a biomimetic three-dimensional scaffold that has been optimized for *in vitro* growth of adherent cells. When the growth matrix is used in the Cytomatrix™ Spinner System, it supports high-density growth of established cell lines, tumor cell lines and heterogeneous primary cell isolates. It is especially valuable for culturing cell types that are difficult to maintain *in vitro* by conventional methods. Matrix units can be removed individually from ongoing cultures for convenient aliquot sampling. The system is particularly well suited for complex cell-cell interaction studies, cell-based assays, and bench-top biomass production.

### **Attribution**

Todd Upton, Ph D, is Director of R&D at Cytomatrix, LLC (Woburn, MA). Telephone (781) 939-0995. Website: [www.cytomatrix.com](http://www.cytomatrix.com). Natalie S. Rudolph, Ph D contributed to the preparation of this manuscript.