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Attaching cells to naturally occurring extracellular matrices prior to cell delivery

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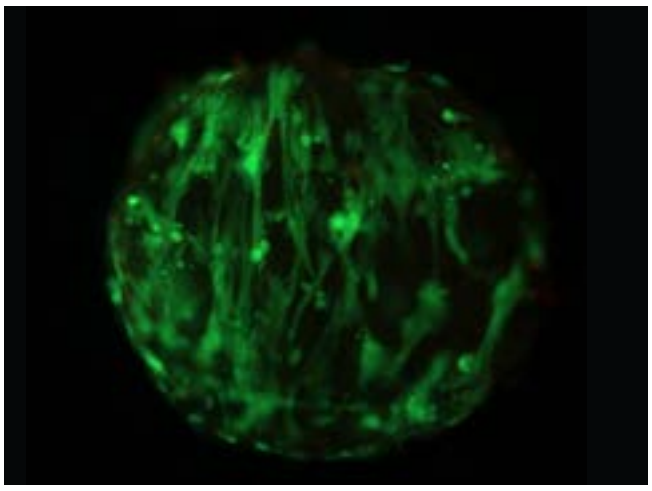

Figure 1. Umbilical cord mesenchymal stem cells grown on a 500 micron SIS disc for 96 hours.

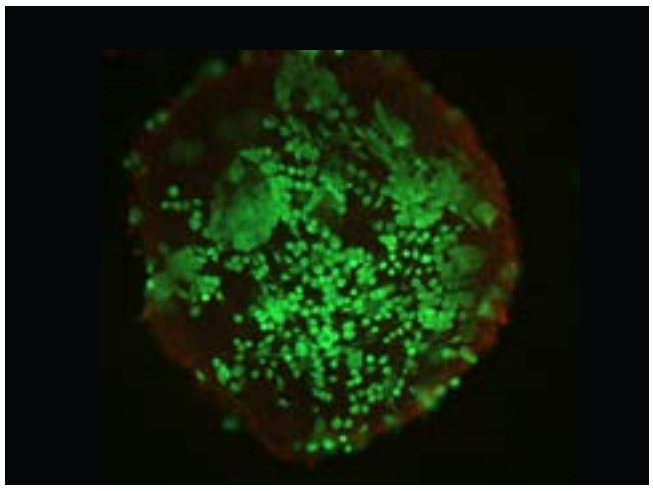

Figure 2. Skeletal muscle-derived cells on a 500 micron SIS disc after 1 hour.

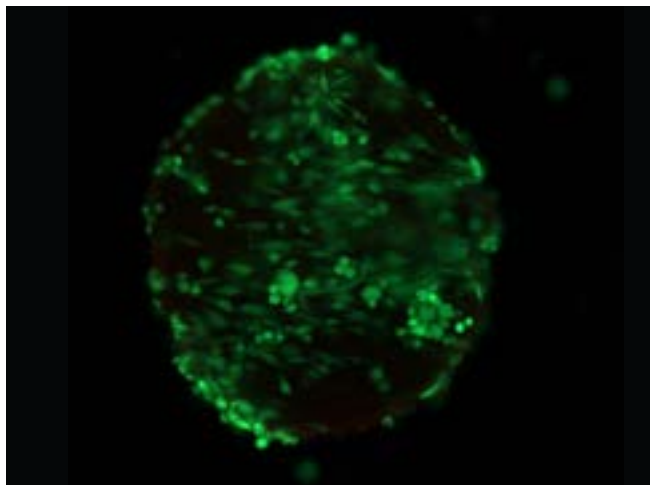

Figure 3. Placental mesenchymal stem cells on a 500 micron SIS disc after 1 hour.

Background

Cell therapy is showing promise for efficacy in the treatment of a variety of conditions (1-3). Yet the almost immediate decline in the number of viable cells after implantation is troublesome (4). The pursuit of a delivery mechanism that increases the survival of cells after implantation and sequestration of cells at the site of delivery has led to investigations utilizing naturally occurring extracellular matrices (ECM), such as **small intestinal submucosa (SIS)**. Adherent-dependent cells, such as mesenchymal stem cells (MSC), undergo anoikis when no attachment sites are available (5). Combining such cells with an ECM containing native cell attachment binding sites should enhance the survival of implanted cells.

SIS is a well-characterized extracellular matrix.
SIS is primarily Type I collagen, but it also contains growth factors, proteoglycans, and glycosaminoglycans. SIS can bind exogenous molecules such as growth factors or pharmaceuticals. In fact, SIS can protect bound growth factors from degradation. Cells that are involved in healing are attracted to SIS *in vitro*. Numerous non-clinical studies describe how SIS enhances healing and angiogenesis in animal models. SIS degrades over time and is replaced with healthy native tissue (6).

SIS is an ideal substrate for cell attachment.
More than 40 cell types, including primary and established cell lines have been grown or attached to SIS (7-12). Cells can attach to SIS via arginylglycylaspartic acid (RGD) peptides rapidly, detectable in 20 minutes. Cells have been grown on sheets of SIS and implanted in various animal models in the fields of urology, orthopedics, gastroenterology, cardiology, plastic reconstruction, dentistry, gynecology, wounds, and hernias (references available upon request).

Cell/ECM combinations have been implanted before, but an injectable version of an ECM would create a less invasive treatment. The data presented here uses an intact, solid-phase configuration of SIS that can be delivered via injection through a needle or catheter (Figure 5). This configuration of SIS maintains the native three-dimensional structure and composition, presenting a healthy microenvironment for attachment-dependent cells.

Attaching cells to SIS for in vitro studies

Several progenitor cell types have been attached to SIS discs: **placental**- and **umbilical cord-derived mesenchymal stem cells** from Cook General Biotechnology, Inc. (Figures 1 and 3); **skeletal muscle-derived cells (skMDC)** from Cook MyoSite (Figure 2); and **endothelial colony forming cells (ECFC)** from Lonza (Figure 6). Cells are maintained in cell culture flasks according to the manufacturers' recommendations. Cells are typically removed from flasks with trypsin/EDTA (Sigma Chemical Co.).

The following attachment protocol was used to attach cells to SIS.

1. Pre-treat SIS discs with complete media for 1 hour.
2. Keep the cell concentration high. For example, combine approximately 50 µL of pre-treated discs and 200 µl of cell suspension containing 1.0 x 10^6 cells (Note: a 50 µL volume of wet discs equates to approximately 4 mg of dry discs).
3. Incubate cells and discs for 1 hour at 37°C to allow for attachment. Cells will start to attach in 20 minutes and approximately 50% of cells will be attached in 1 hour.
4. If there are attachment issues, incubation can be done with gentle mixing, for example rotation.
5. Unattached cells can be gently washed away for assays.
6. Live/Dead (Invitrogen, catalog #L3224) has been used to visualize cells. SIS (primarily collagen) will autofluoresce in the range of 305-450 nm.

Growing cells on SIS discs in multi-well tissue culture plates can be done for longer periods of time (Figure 1, 96 hr). Cells can be seen on SIS discs as soon as 10 minutes after combining. Initially, the cells are round, appearing to contain high levels of Calcein AM (Figure 3). But as the cells attach, they flatten and elongate, with cellular extensions visible (Figure 4). Cells will attach to both sides of the SIS disc and therefore it can be difficult to capture all attached cells in one plane of focus (Figure 5). If clumps of cells are present in the cell suspension, clumps will also attach to the SIS (Figure 3). Cells will also attach to the edge of the SIS (Figure 3). As the cells grow, some varieties can appear to align with the collagen filaments (Figure 1), whereas other cell types will produce a monolayer.

Measuring the attachment of cells to SIS

The protocol for attaching cells to SIS was followed. The cell concentration was determined prior to combining the cells and SIS in Step 3 and then again after Step 3. The percentage of cells attached was calculated as follows:

$$\text{Percent Attached} = 100 - \left(\frac{\text{End Concentration}}{\text{Starting Concentration}}\right) \times 100$$

Each cell type has a different attachment profile. When cells were detached from treated cell culture flasks with trypsin/EDTA, **27-55% of the cells were attached in one hour** (Figure 4).

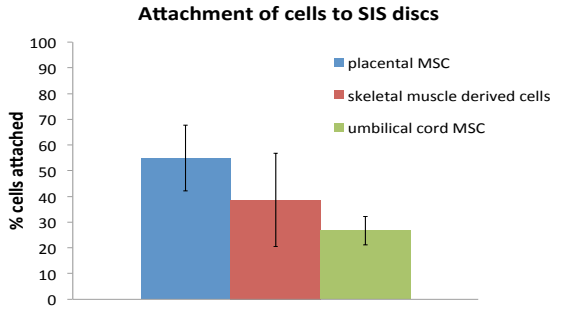

Figure 4. Attachment of cells to SIS. Approximately 4 mg of dry SIS discs were pretreated with complete media for one hour followed by the addition of 1 x 10^6 cells in 200 µl of media. The cell concentration in the solution was determined after a 1 hour incubation at 37°C with gentle agitation. The percentage of cells attached was calculated as above. The means of several assays were combined and the bars are standard deviation. For placental MSC n = 8 and for skeletal muscle derived cells and umbilical cord MSC n = 3.

In addition to detaching cells from culture flasks with trypsin/EDTA, removing the divalent cations (incubating in the presence of calcium- and magnesium-free PBS) will also allow cells to detach. This allows the initial attachment to SIS to occur quicker, but does not appear to increase the total number of cells that attach over time. The pretreatment of the SIS can be done in media containing 5-10% fetal bovine serum (FBS; Sigma Chemical Co.) or 5% **human platelet lysate** (HPL; Cook General BioTechnology).

Figure 5: Vial of 200 micron SIS discs, photos and SEM images of individual 200 micron SIS discs.

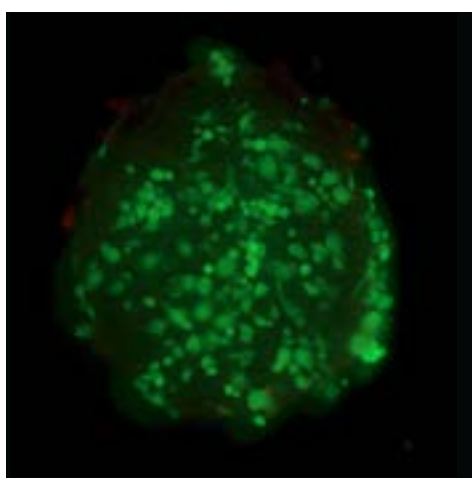

Figure 6: Endothelial colony forming cells (ECFC) on a 500 micron SIS after 1 hour of incubation.

Summary

- SIS is a clinically proven extracellular matrix material that enhances healing.
- Cells rapidly attach to SIS.
- SIS binds, protects, and presents growth factors (13).
- An injectable version of SIS can be used to deliver cells in vivo (experiments ongoing).
- A dosing example for in vivo delivery might be 250 µl containing 2.5-5.0 x 10^5 attached cells on 2,000 SIS discs. Unattached cells can be present in this dose.

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