of plastic petri dishes and the cells seeded on the surface of the gels in sufficient medium to completely cover the cells.

When rat keratinocytes were seeded onto Vitrogen 100 bovine dermal collagen, which is composed of 95% type I and 5% type II collagens, firm attachment to this substratum resulted (24). However, 3 days after seeding, growth resulted in clusters of cells rather than in a uniform proliferation over the substratum surface as seen with plastic substrata. At this time, holes were also produced in the collagen gel, and the surface began to dissolve. If the culture was incubated longer, the entire collagen layer disappeared, and the cells were seen growing directly on the plastic surface of the container.

When rat tail collagen was used as a substratum for the growth of rat keratinocytes, attachment was observed 6 hr after plating, compared with 18 to 24 hr on commercial plastic vessels (24). By 24 hr, 75% of the surface of this substratum was covered with keratinocytes that had spread and proliferated. A confluent monolayer was formed in 4 days. However, gels formed from this collagen are very soft and difficult to handle.

Gels formed from mixtures of Vitrogen 100 and rat tail collagen were also investigated as appropriate substrata (24). A mixture containing equal amounts of the two types (1:1, vol/vol) supported attachment and growth of rat keratinocytes as well as rat tail collagen alone, and also resulted in a firm substratum that would allow relocation of the entire culture to the air–medium interface (a procedure that will be described in a later section). This collagen mixture also supported stratification and differentiation of submerged keratinocytes to a greater extent than plastic substrata.

3. *Synthetic Membranes as Substrata*

In a concentrated study, 11 commercially available and experimental synthetic membrane filters were selected and evaluated as appropriate substrata for rat keratinocytes (25). In Table 1, the source of each membrane and the material from which it was constructed are listed. Membranes TCM200, TCM440, HA-TF, and RA-TF were selected for study because they are specifically treated to remove detergent and are more suitable for tissue culture use. The Acroshield D (ACRO) and silicone–polycarbonate copolymer (MEM) were included because they are transparent and would allow cell growth to be continuously monitored by phase-contrast microscopy. All membranes used in this particular study were 13 mm in diameter and were placed in the wells of 24-well plastic culture vessels before seeding a suspension of keratinocytes on their surfaces. Wells without membranes were also seeded and served as a basis for comparing the resulting attachment and growth of cells on each membrane (plastic controls).