second membrane into the third fluid, where they form vesicles. The size of the vesicles may be adjusted with the pore size and pressure.

[**0085**] Fluids may flow through fluid paths **20**, **30** under constant flow conditions, varying flow conditions, or even conditions of no flow depending on the interaction to be performed. Where it is desired to flow a fluid through flow paths **20**, **30**, flow may be provided by pressure, such as by pumps, columns of water, and the like, by electroosmosis, or any other mechanism capable of generating a desired flow condition. As an alternate example, fluid may be supplied at a constant volumetric flow rate or a volumetric flow rate that varies over time in a predetermined or random manner, supplying fresh material to contact region **40** at a constant or variable rate. Such a system may allow a reaction or test to be run continuously, which is not possible in a tray system. Whatever the flow conditions, it is generally preferred to equalize the pressure across contact regions so that convection is minimized and the risk of damaging the convection controller is reduced. As discussed in U.S. Provisional Patent No. 60/286,476, which is hereby incorporated by reference in its entirety, the behavior of flows in microfluidic fluid paths is complex and depends, for example, on the aspect ratio of the fluid paths. At relatively high aspect ratios, where flow conditions are laminar and there is no pressure difference across the fluid contact region, no convection controller may be required as the only exchange between fluid paths may be diffusive. However, as these conditions may be difficult to attain, some sort of convection controller is preferred. In some embodiments, it may be desired to maintain a pressure gradient between fluid paths, such as where it is desired to provide a constant flow of material from one fluid path into the other. In such an instance, a small amount of convection through the convection controller is intended and desired.

[**0086**] Where it is desired not to flow a fluid through one or more of fluid paths **20**, **30**, an immobilizer may be used. The immobilizer may be any material that holds an interaction material in such a way that the material can interact with another interaction material and is not active in the context of the interaction. For example, the immobilizer may be an inert matrix. In one embodiment, the immobilizer is a thixotropic material, such as a flowable gel that is capable of being flowed at sufficient pressure, but that generally does not flow. In another embodiment, the immobilizer is a flowable material that is capable of being transformed into a non-flowable material by curing, setting, or the like. An example of such a material is agarose. Interaction materials, such as test materials and indicator materials may be introduced into the immobilizer (or part of it) and immobilized with it. In another embodiment, a fluid that is not an immobilizer but from which an interaction material may be deposited by precipitation, adsorption, absorption, covalent bonding or other chemical reaction, or the like may be used instead of an immobilizer to immobilize an interaction material. An example of such a material is a solution of a protein, from which the protein may be deposited via adsorption. While an immobilizer will typically remain within the fluid path, the fluid in this embodiment would typically be removed after the interaction material has deposited as desired. Immobilization may be reversible. For example, a thixotropic immobilizer may have sufficient pressure applied to induce flow. As another example, precipitation by adsorption, or the like, may be reversible. Immobilization may also be substantially irreversible. This may allow, for example, a patterned material to remain attached to a substrate while a material it interacted with is removed from the patterned material/and or substrate, potentially resulting in reusable devices.

[**0087**] In an embodiment where the immobilizer will be left in the fluid path, it may be preferred that the immobilizer allows diffusion through it so that interaction material immobilized therein may travel to the contact region. While the immobilizer would typically fill fluid path **20**, **30**, this is not required and as little of fluid path **20**, **30** needs be filled as is required to contact the interaction material to the contact region. For example, at least 5% of the fluid path, at least 25% of the fluid path, or at least 50% of the fluid path at any given point may be filled with the immobilizer. In some embodiments, both fluid paths **20**, **30** may contain an immobilizer.

[**0088**] It should be appreciated that once a material is immobilized in a fluid path, the fluid path can be removed from the material, or opened on at least one side. For example, where a fluid path has three sides formed of a monolith and one side that is a separate material, (a substrate), fluid may be immobilized therein and the monolith removed, leaving a pattern of immobilized material on the substrate.

[**0089**] The use of immobilization may also allow use of only a single fluid path, or set of fluid paths which do not cross or otherwise make contact with one another at contact points. For example, instead of immobilizing a material of interest in a fluid path and allowing it to interact at a contact point through a convection controller, the material can be immobilized on, or in, a substrate. The substrate may then be used to form one side of a fluid path and fluid may be flowed across it to allow the desired interaction. The material to be immobilized may be patterned on to the substrate such that it only comes into contact with, and is able to interact with, a fluid flowing in the fluid path in certain locations. For example, the material may be patterned in a way that provides contact points similar to those provided at convection inhibitors in crossing fluid paths.

[**0090**] One embodiment where only a single fluid path, or set of fluid paths which do not cross or otherwise make contact with one another, are used is illustrated in FIGS. **17** (set of fluid paths) and **19** (single fluid path). In the embodiment illustrated in **FIG. 19**, which is a microfluidic system **10**, a series of parallel lines of interaction material **200** are patterned onto a substrate **202** with a series of parallel fluid paths **204** crossing them at a right angles, creating multiple interaction sites **206**. As is the case with crossing fluid paths, this arrangement allows the number of interaction sites to increase geometrically with a linear increase in the number of fluid paths and lines of interaction material. Accordingly, it will be recognized that this embodiment may be used in many of the same testing and other interaction applications disclosed previously.

[**0091**] Where a substrate **202** is used, it may be any material that is able to have a pattern formed thereon, or therein, of the desired interaction material. For example, the substrate may be a membrane or may be a thicker piece of material. In some embodiments, the substrate may be constructed from a material that would be suitable for use as a convection controller. Where the interaction material is a