

The solutions of tetra-amino PEG and tetra SE-PEG were mixed together with a pellet containing human skin fibroblast ("HSF") cells (CRL #1885, passage 4, obtained from American Tissue Type Culture Collection, Rockville, Md.). Two hundred fifty (250) microliters of the resulting cell-containing tetra-amino PEG/tetra SE-PEG (PEG-PEG) solution was dispensed into each of two wells on a 48-well culture plate and allowed to gel for approximately 5 minutes at room temperature. One (1) milliliter of Dulbecco Modified Eagle's Media (supplemented with 10% fetal bovine serum, L-glutamine, penicillin-streptomycin, and non-essential amino acids) was added to each of the two wells. The concentration of cells was approximately 3×10^5 cells per milliliter of tetra-amino PEG/tetra SE-PEG solution, or 7.5×10^5 cells per well.

To prepare a control, a pellet of HSF cells were suspended in 1.2 ml of complete media. Two hundred fifty (250) microliters of the control mixture was dispensed into each of three wells on the same 48-well culture plate as used above. Each well was estimated to contain approximately 7.5×10^5 cells. Each well was given fresh media every other day.

Initially, the cell-containing tetra-amino PEG/tetra SE-PEG gels were clear and the cells were found to be densely populated and spheroidal in morphology, indicating that there was little adhesion between the cells and the PEG/PEG gel (the cells would normally assume a flattened, spindle-shaped morphology when adhered to a substrate, such as to the treated plastic of the tissue culture plates). After three 3 days incubation at 37° C., the media in the wells containing the PEG/PEG gels was found to have lightened in color (Dulbecco Modified Eagle's Media is normally red in color), indicating a pH change in the media. This indicated that the cells were alive and feeding. At 7 days incubation at 37° C., the cells were still spheroidal in morphology (indicating lack of adhesion to the gel) and the media had lightened even further, indicating that the cells were still viable and continued to feed.

On day 7, the contents of each well were placed in a 10% formalin solution for histological evaluation. According to histological evaluation, an estimated 75% of the cells in the wells containing the PEG/PEG gels appeared to be alive, but did not appear to be reproducing.

The results of the experiment indicate that HSF cells are viable in the tetra-amino PEG/tetra SE-PEG crosslinked gels, but did not seem to adhere to the gel and did not appear to reproduce while entrapped within the gel matrix. As described above, adherence or non-adherence of cells to a substrate material can influence the cells' morphology. In certain types of cells, cellular morphology can, in turn, influence certain cellular functions. Therefore, non-adherence of the cells to the PEG-PEG gel matrix may be an advantage in the delivery of particular cell types whose function is influenced by cell morphology. For example, the ability of cartilage cells to produce extracellular matrix materials is influenced by cellular morphology: when the cells are in the flattened, spindle-shaped configuration, the cells are in reproductive mode; when the cells are in the spheroidal configuration, reproduction stops, and the cells begin to produce extracellular matrix components.

Because the PEG-PEG gels are not readily degraded in vivo, the gels may be particularly useful in cell delivery applications where it is desirable that the cells remain entrapped within the matrix for extended periods of time.

What is claimed is:

1. A method for preparing a negatively charged compound-containing matrix useful for delivery of a nega-

tively charged compound to a mammalian subject, comprising the steps of:

providing a first synthetic polymer containing nucleophilic groups and a second synthetic polymer containing electrophilic groups;

forming a mixture by mixing the first synthetic polymer and the second synthetic polymer to initiate crosslinking, wherein the first synthetic polymer is present in the mixture in molar excess compared to the second synthetic polymer;

allowing the first synthetic polymer and the second synthetic polymer to continue crosslinking to form a positively charged crosslinked synthetic polymer matrix; and

reacting the matrix with the negatively charged compound.

2. The method of claim 1, wherein said first synthetic polymer has m nucleophilic groups and said second synthetic polymer has n electrophilic groups, wherein m and n are each greater than or equal to 2, and wherein m+n is greater than or equal to 5.

3. The method of claim 1, wherein the first synthetic polymer is a polyethylene glycol, and wherein the nucleophilic groups are selected from a primary amino group and a thiol group.

4. The method of claim 1, wherein the second synthetic polymer is a polyethylene glycol derivative, and wherein the electrophilic groups are succinimidyl groups.

5. The method of claim 1, wherein the negatively charged compound is succinylated collagen.

6. The method of claim 1, wherein the negatively charged compound is a negatively charged glycosaminoglycan derivative selected from the group consisting of: sodium hyaluronate, keratan sulfate, keratosulfate, sodium chondroitin sulfate A, sodium dermatan sulfate B, sodium chondroitin sulfate C, heparin, esterified chondroitin sulfate C, esterified heparin, and combinations thereof.

7. A method for preparing a positively charged compound-containing matrix useful for delivery of a positively charged compound to a mammalian subject, comprising the steps of:

providing a first synthetic polymer containing nucleophilic groups and a second synthetic polymer containing electrophilic groups;

forming a mixture by mixing the first synthetic polymer and the second synthetic polymer to initiate crosslinking, wherein the second synthetic polymer is present in the mixture in molar excess compared to the second synthetic polymer;

allowing the first synthetic polymer and the second synthetic polymer to continue crosslinking to form a negatively charged crosslinked synthetic polymer matrix; and

reacting the matrix with the positively charged compound.

8. The method of claim 7, wherein said first synthetic polymer has m nucleophilic groups and said second synthetic polymer has n electrophilic groups, wherein m and n are each greater than or equal to 2, and wherein m+n is greater than or equal to 5.

9. The method of claim 7, wherein the first synthetic polymer is a polyethylene glycol, and wherein the nucleophilic groups are selected from a primary amino group and a thiol group.

10. The method of claim 7, wherein the second synthetic polymer is a polyethylene glycol, and wherein the electrophilic groups are succinimidyl groups.