biodegradable scaffolds made of natural or synthetic polymers, the problem of diffusion limitation prevails as nutrients from the culture media are not able to efficiently reach or perfuse the cells attached on the scaffolds.

In view of these and other deficiencies in currently existing techniques, there is a need for new methods of engineering micropatterned three-dimensional constructs for the seeding of cells.

Summary ~~of the Invention~~

The present disclosure~~invention~~ relates to a two-photon technology capable of building high-resolution three-dimensional tissue constructs. The technology provides a simple and flexible method for producing microstructures leading to cell growth in three-dimensional cell culture and tissue engineering.

The present disclosure~~In a first aspect, the invention~~ provides a method for producing a three-dimensional scaffold construct comprising encapsulated cells, the method comprising:

(a) providing a solution comprising cells to be encapsulated, a photoinitiator, and a plurality of units capable of forming polymer chains;

(b) providing a photolithography instrument comprising a two-photon laser; and

(c) using the instrument to apply the laser to the solution to activate the photoinitiator thereby facilitating polymerisation of said units to form polymer chains, and, cross-linking of the polymer chains;

wherein the laser is applied to the solution in three-dimensions in a pre-defined pattern to assemble said construct, and said cells are encapsulated within the assembled construct.

In a first aspect, there is provided a method for producing a three-dimensional scaffold construct comprising encapsulated cells, the method comprising: (a) providing a solution comprising cells to be encapsulated, a photoinitiator, and a plurality of units capable of forming polymer chains; (b) providing a photolithography instrument comprising a two-photon laser; (c) using the instrument to apply the laser to the solution to activate the photoinitiator thereby facilitating polymerisation of said units to form polymer chains, and, cross-linking of the polymer chains; wherein the laser is applied to the solution in three-dimensions in a pre-defined pattern to assemble said construct, and said cells are encapsulated within the assembled construct; and (d) culturing the construct of (c) comprising the encapsulated cells.

The present disclosure~~In a second aspect, the invention~~ provides a method for producing a three-dimensional scaffold construct comprising encapsulated cells, the method comprising:

providing a solution comprising cells to be encapsulated, a photoinitiator, and either or both of:

(a) a plurality of units capable of forming polymer chains,

(b) a plurality of polymer chains;

providing a photolithography instrument comprising a two-photon laser; and

using the instrument to apply the laser to the solution to activate the photoinitiator thereby facilitating polymerisation of said units and/or polymer chains, and cross-linking of said polymer chains;

wherein the laser is applied to the solution in three-dimensions in a pre-defined pattern to assemble said construct, and said cells are encapsulated within the assembled construct.

In one embodiment of the first ~~and second~~ aspects, the scaffold construct is assembled according to a three dimensional computer assisted design (CAD) image that is read by said photolithography instrument.

In one embodiment of the first ~~and second~~ aspects, the cells are encapsulated during cross-linking of the polymer chains in three dimensions.

In one embodiment of the first ~~and second~~ aspects, the cells are encapsulated by cross-linking of the polymer chains in three dimensions.

In one embodiment of the first ~~and second~~ aspects, the laser emits energy in the infrared region.

In one embodiment of the first ~~and second~~ aspects, the cells comprise human umbilical vascular endothelial cells (HUVEC).

In one embodiment of the first ~~and second~~ aspects, the cells comprise hepatocytes.

In one embodiment of the first ~~and second~~ aspects, the cells comprise stem cells.

In one embodiment of the first ~~and second~~ aspects, the construct comprises more than one type of polymer chain.

In one embodiment of the first ~~and second~~ aspects, the unit is monomer of a resin polymer.

In one embodiment of the first ~~and second~~ aspects, the unit is a fibrillar protein.

In one embodiment of the first ~~and second~~ aspects, the fibrillar protein is fibrinogen.

In one embodiment of the first ~~and second~~ aspects, the photoinitiator is ruthenium II trisbipyridyl chloride $[RuII(bpy)_3]^{2+}$, and the solution comprises an oxidising agent.

In one embodiment of the first ~~and second~~ aspects, the oxidising agent is sodium persulfate.

In one embodiment of the first ~~and second~~ aspects, the construct is ring-shaped.

In one embodiment of the first ~~and second~~ aspects, the pores are between about 1μm and about 50 μm in width or diameter.

In one embodiment of the first ~~and second~~ aspects, the pores are between about 1 μm and about 10μm in width or diameter.

In one embodiment of the first ~~and second~~ aspects, the method further comprises washing the construct to substantially remove non-crosslinked polymer chains and non polymerised units.

In one embodiment of the first ~~and second~~ aspects, the polymer chains are biodegradable.

In one embodiment of the first ~~and second~~ aspects, the solution further comprises a bioactive component.

In one embodiment of the first ~~and second~~ aspects, the cells are in the solution at a concentration of between about 1 x 10^6/ml and about 1 x 10^7/ml.

In one embodiment of the first ~~and second~~ aspects, the method further comprises seeding additional cells to the construct after completion of said polymerization and cross-linking.

In one embodiment of the first ~~and second~~ aspects, the ring-shaped construct has a diameter of about 400μm, and a thickness of about 100μm.

The present disclosure~~In a third aspect, the invention~~ provides a scaffold construct produced in accordance with the method of the first aspect ~~or the second aspect~~.

Brief Description of the Drawings

Preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying figures wherein:

Figure 1 *is a graph illustrative of degradation of cross-linked fibrin in media containing* (◄) Tris buffer only (control), and (■) 0.1 μg/ml, (●) 1.0 μg/ml, (▲) 10 μg/ml, (▼) 50 μg/ml over 24 days.

Figure 2 provides light microscopy images of HUVECs seeded on fibrin surface after (A) 24 h and (B) 48 h. Cells were stained with the Live/Dead® assay.

Figure 3 is a graph illustrative of the effect of $[Rull(bpy)_3]^{2+}$ concentration on the viability of HUVECs. Absorbance of the MTT assay was determined at 490 nm.

Figure 4 provides light microscopy images of (**A, C**) the fibrin constructs with cells stained with (**A, C**) the Live/Dead® assay and (**B, D**) the EthD-1 component of the Live/Dead® assay. Cells in the background represent those that were not washed away and remained attached onto the cover slip. (**A**) Images of four scanned devices on a cover slip showing rings of live cells grown on the fibrin constructs. (**B**) Image taken from the channel to view EthD-1 fluorescence in (**A**). The fibrin constructs display auto-fluorescence, giving the false appearance of a ring of dead cells. (**C**) Magnified image of (**A**) showing one of the constructs. (**D**) Image taken from channel to view EthD-1 fluorescence in (**C**), showing the auto-fluorescence of the fibrin construct.

Figure 5 provides (**A**) Bright-field image of the fibrin construct. HUVECs encapsulated within the scaffold were slightly visible. The brown lines depict the way the