CLAIMS:

1. A drop on demand 3D printed hydrogel formed from a maleimide containing polymer selected from a PEG maleimide, a gelatin maleimide, or a combination thereof, cross-linked using a bis-thiol containing cross-linking agent having at least two thiol functional groups, wherein the hydrogel is formed within 1 second or less from printing of the maleimide containing polymer and the cross linking agent, and wherein the pH of the polymer and/or cross-linking agent is adjusted with NaOH or other strong base.

2. The 3D printed hydrogel according to claim 1, wherein the maleimide containing polymer comprises a PEG maleimide.

3. The 3D printed hydrogel according to claim 1, wherein the maleimide containing polymer comprises a gelatin maleimide.

4. The 3D printed hydrogel according to any one of claims 1 to 3, wherein the bis-thiol containing cross-linking agent is selected from the group consisting of synthetic polymers, biopolymers, small molecules, bioactive molecules, and any combination thereof.

5. The 3D printed hydrogel according to claim 4, wherein the bis-thiol containing cross-linking agent is selected from PEG-bis-thiol, matrix metalloproteinase (MMP) responsive peptides, or a combination thereof.

6. The 3D printed hydrogel according to claim 5, wherein the bis-thiol containing cross-linking agent is a PEG-bis-thiol.

7. The 3D printed hydrogel according to claim 5, wherein the bis-thiol containing cross-linking agent is a matrix metalloproteinase (MMP) responsive peptide.

8. The 3D printed hydrogel according to claim 7, wherein the MMP-responsive peptide is an MMP-responsive bis-cysteine peptide.

9. The 3D printed hydrogel according to claim 8, wherein the MMP-responsive peptide is selected from the group consisting of GCIPVSLRSGCG, GCRDGPQGIWGQDRCG, GCRDPLGLDRCG, GCRDEAPLKQDRCG, and any combination thereof.

10. The 3D printed hydrogel according to claim 9, wherein the MMP-responsive peptide is GCIPVSLRSGCG, GCRDPLGLDRCG, or a combination thereof.

11. The 3D printed hydrogel according to claim 2, wherein the maleimide containing polymer is PEG-maleimide substituted with at least one bioactive molecule selected from RGD, YIGSR and IKVAV.

12. The 3D printed hydrogel according to claim 3, wherein the maleimide containing polymer is gelatin maleimide and the bis-thiol containing cross-linking agent is PEG-bis-thiol.

13. The 3D printed hydrogel according to any one of claims 1 to 12, wherein the molar ratio of maleimide containing polymer to bis-thiol containing cross-linking agent is in the range of 10:1 to 1:10.

14. The 3D printed hydrogel according to any one of claims 1 to 13 containing cells.

15. The 3D printed hydrogel according to claim 14, wherein the cells are suspended within a part of the hydrogel or the cells are substantially uniformly suspended throughout the hydrogel.

16. The 3D printed hydrogel according to claim 14 or 15, wherein the concentration of printed cells is in the range of 1×10^5 to 5×10^8 cells/mL.

17. The 3D printed hydrogel according to any one of claims 14 to 16, wherein the cells are selected from the group consisting of liver cells, gastrointestinal cells, pancreatic cells, kidney cells, lung cells, tracheal cells, vascular cells, skeletal muscle cells, cardiac cells, skin cells, smooth muscle cells, connective tissue cells, corneal cells, genitourinary cells, breast cells, reproductive cells, endothelial cells, epithelial cells, fibroblast, neural cells, Schwann cells, adipose cells, bone cells, bone marrow cells, cartilage cells, pericytes, mesothelial cells, cells derived from endocrine tissue, stromal cells, stem cells, progenitor cells, lymph cells, blood cells, endoderm-derived cells, ectoderm-derived cells, and mesoderm-derived cells, and any combination thereof.

18. The 3D printed hydrogel according to claim 1 further comprising a bioactive molecule.

19. The 3D printed hydrogel according to claim 18, wherein the bioactive molecule is a free bioactive molecule or bound to the maleimide containing polymer, the bis-thiol containing cross-linking agent, or both the maleimide containing polymer and the bis-thiol containing cross-linking agent.

20. The 3D printed hydrogel according to claim 18 or 19, wherein the bioactive molecule is selected from the group consisting of a peptide, MMP-responsive peptide, protein, polysaccharide, drug, therapeutic agent, antibody, small molecule inhibitor, kinase inhibitor, phosphatase inhibitor, antigen, pathogen, platelet, growth factor, cytokine, amino acid, nutrient, conditioned media, antibiotic, antiviral, RNA, and any combination thereof.

21. The 3D printed hydrogel according to claim 20, wherein the bioactive molecule is collagen.

22. The 3D printed hydrogel according to any one of claims 1 to 21, further including a cell culture medium.

23. The 3D printed hydrogel according to any one of claims 1 to 22, wherein the pH of the maleimide containing polymer, the cross-linking agent, or both the maleimide containing

polymer and the cross-linking agent is adjusted to a pH of 6.5 to 7.5 prior to printing using NaOH or other suitable base.

24. The 3D printed hydrogel according to claim 23, wherein the pH of the maleimide containing polymer, the cross-linking agent, or both the maleimide containing polymer and the cross-linking agent is adjusted to about pH 7.4 prior to printing, wherein the pH is adjusted using NaOH or other suitable base.

25. A method of preparing a drop on demand 3D printed hydrogel, the method comprising:

providing a polymer bio-ink comprising a maleimide containing polymer selected from a PEG maleimide, a gelatin maleimide, or a combination thereof;

providing an activator comprising a bis-thiol containing cross-linking agent having at least two thiol functional groups;

adjusting the pH of the polymer bio-ink and/or activator with NaOH or other strong base;

printing the polymer bio-ink and the activator by drop on demand printing to prepare the printed hydrogel within 1 second, wherein a drop of polymer bio-ink is applied to a substrate followed by a drop of activator to form the hydrogel, or a drop of activator is applied to the substrate followed by a drop of polymer bio-ink to form a hydrogel droplet; and

repetitive deposition of a drop of the polymer bio-ink and the activator onto the hydrogel droplet to form a 3D printed hydrogel.

26. The method according to claim 25, further comprising providing cells during printing to form a 3D printed hydrogel containing cells.

27. The method according to claim 26, wherein cells are present in the polymer bio-ink, in the activator, both in the polymer bio-ink and the activator, or in a separate medium prior to 3D printing.

28. The method according to any one of claims 25 to 27, wherein the polymer bio-ink, the activator, or both the polymer bio-ink and the activator are adjusted to about pH 7.4 prior to printing.

29. A cell assay comprising a 3D printed hydrogel containing cells according to any one of claims 14 to 17.

30. Use of a 3D printed hydrogel containing cells according to any one of claims 14 to 17 for a cell assay.