**Supplementary Information**

# Self-Glucose Feeding Hydrogels by Enzyme Empowered Degradation for 3D Cell Culture

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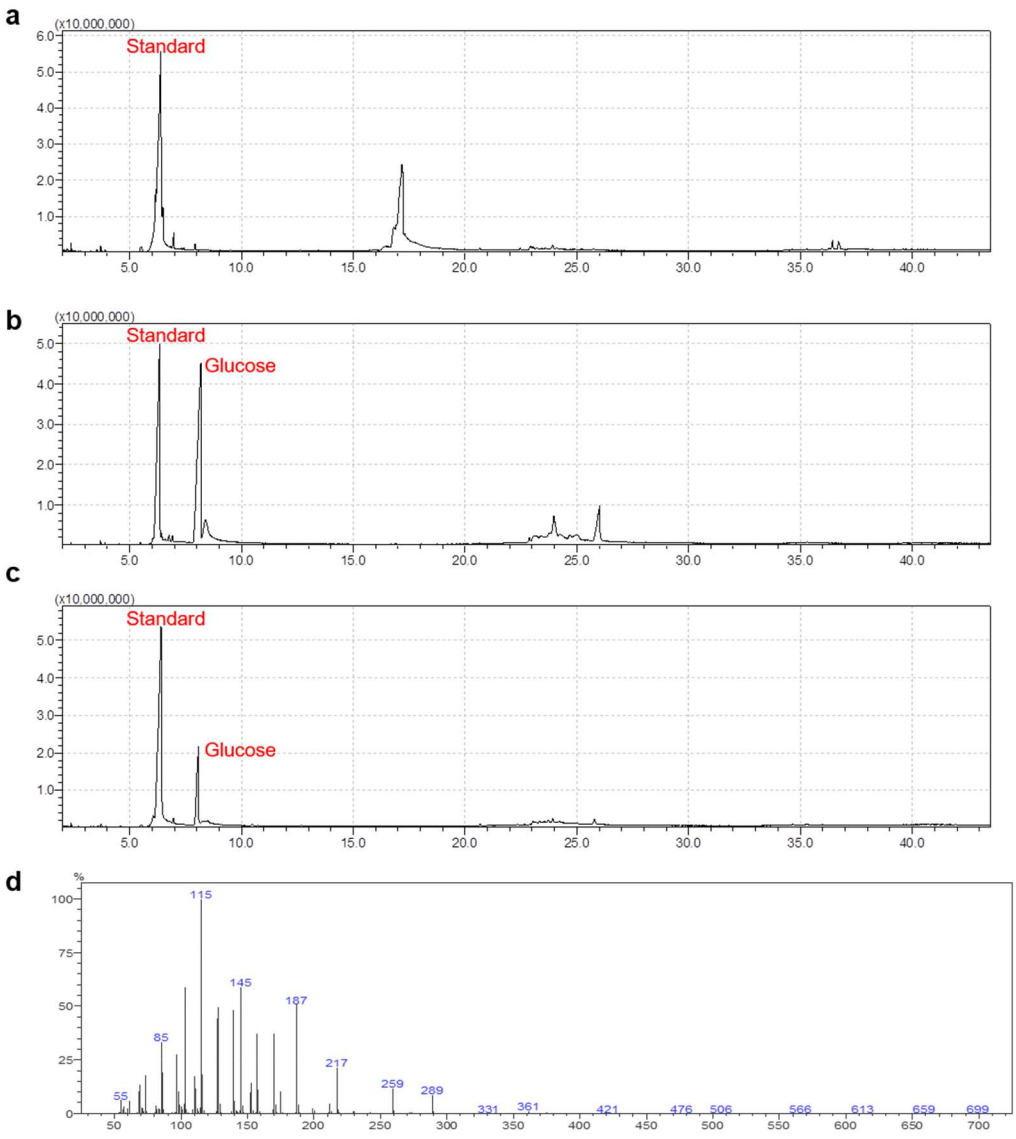


**Figure S1.** Hydrolysis of LMeLam in solution using different enzymes. Representative of LMeLam incubated with a. 21% (v/v) LM b. 0.7% (w/v) of GA c. Combination of GA (0.7% w/v) and LM (21% v/v) for 4 days at 37°C. d. Mass spectrum of residual glucose obtained from degradation.

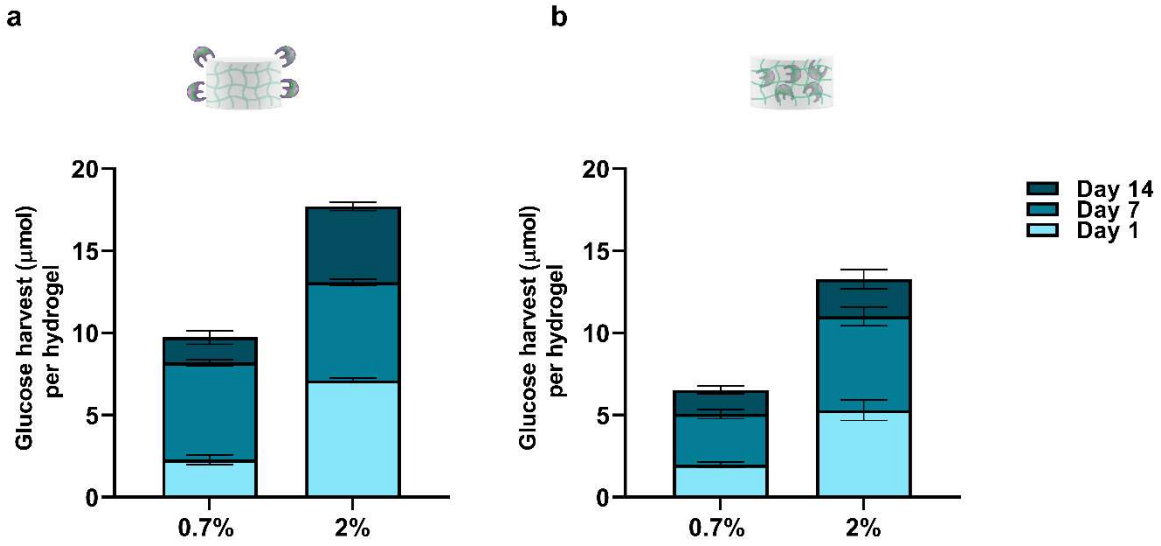


**Figure S2**. Glucose harvest in unit size (µmol) per hydrogel **a**. Degradation from outside **b**. Degradation from inside (enzyme encapsulated) at day 1,7 and 14.

**Table S1**.Degradation products of GA-encapsulated hydrogels over two weeks.

| Glucoamylase | Hydrolysis Products | Peak Area (%) | Total glucose harvest (µmol) |
|---|---|---|---|
| 0.7% | Monosaccharide | 96.5 ± 2.06 | 6.53 ± 0.83 |
| | Trisaccharide | 2.42 ± 2.00 | 0.16 ± 0.02 |
| | Disaccharide | 1.08 ± 0.09 | 0.07 ± 0.009 |
| 2% | Monosaccharide | 99.4 ± 0.32 | 13.26 ± 1.89 |
| | Trisaccharide | 0.39 ± 0.30 | 0.05 ± 0.007 |
| | Disaccharide | 0.33 ± 0.20 | 0.04 ± 0.006 |

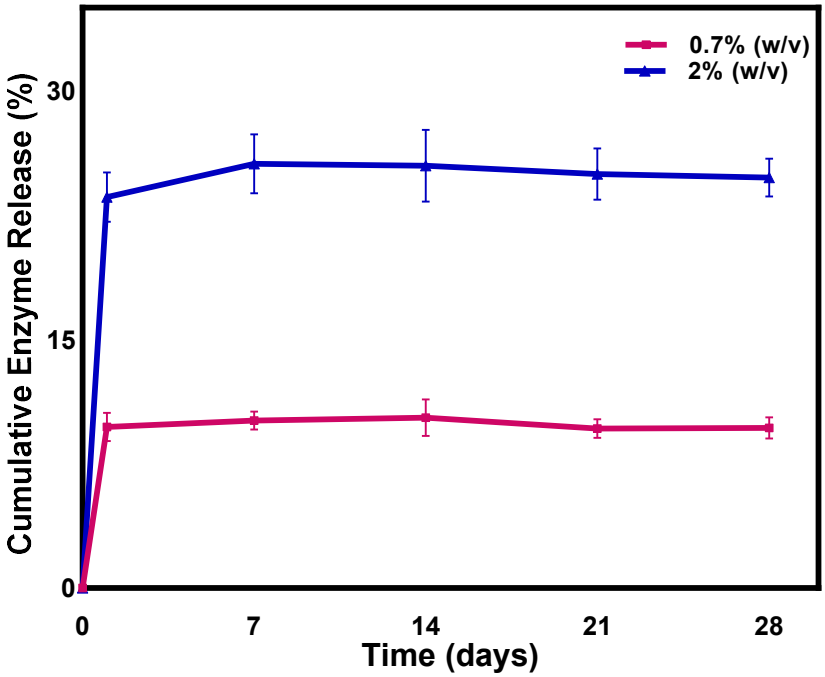


**Figure S3.** Characterization of GA-encapsulated laminaran hydrogels. GA leach out profile over 28 days incubation in PBS at 37°C. Error bars (small and barely visible) represent S.D (n=8).

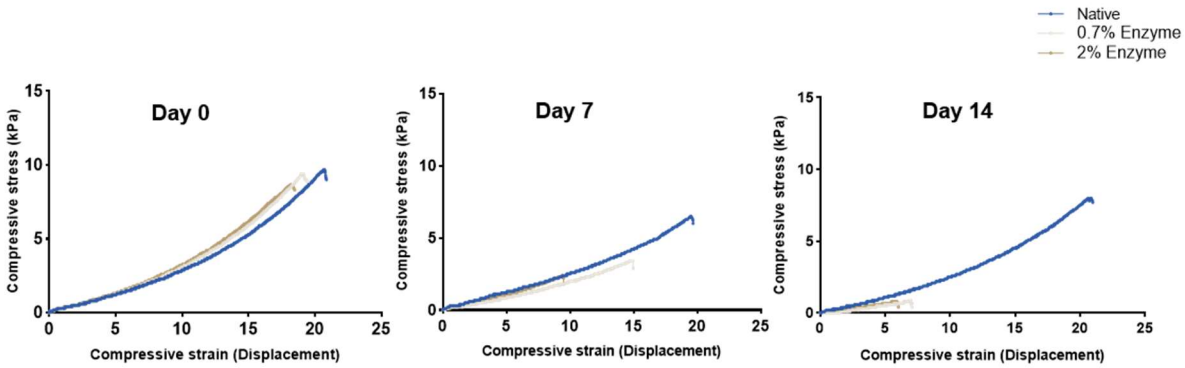


**Figure S4.** Characterization of mechanical properties of GA-encapsulated hydrogels. Representative compressive stress–strain curves for enzyme encapsulated and native laminaran hydrogels in day 0,7 and 14 (from left to right).

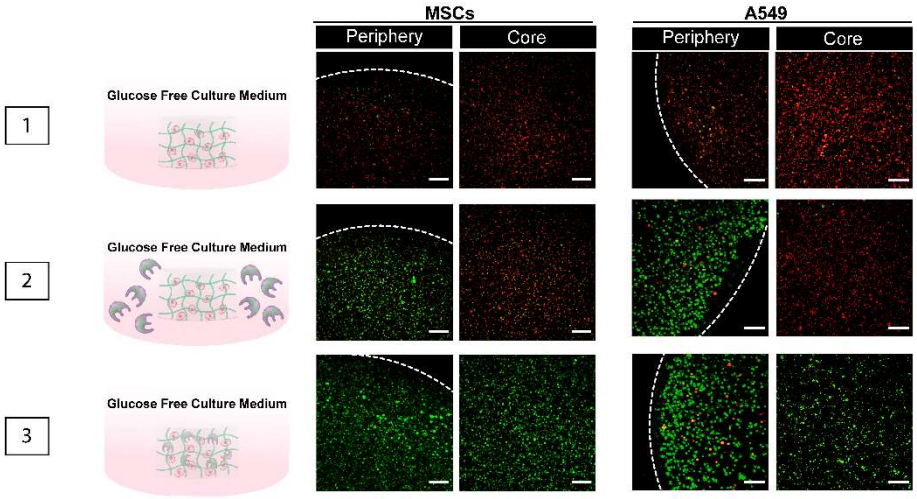


**Figure S5**. Representative fluorescence images of Live/Dead assay for MSCs and A549 cells in the periphery and core of the hydrogels after 14days of culture while 1. no glucose was available for cells 2. Glucose was provided through degradation of hydrogel from the outside degradation and 3. Glucose was produced from inside the hydrogel by encapsulation 0.7% GA.

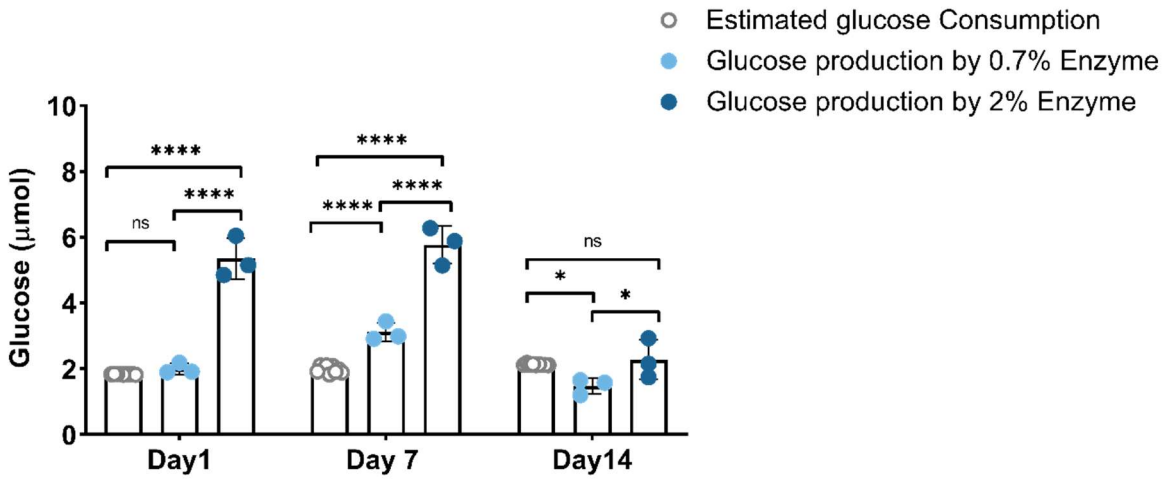


**Figure S6.** Glucose consumption of MSCs vs glucose produced via enzymatic degradation.

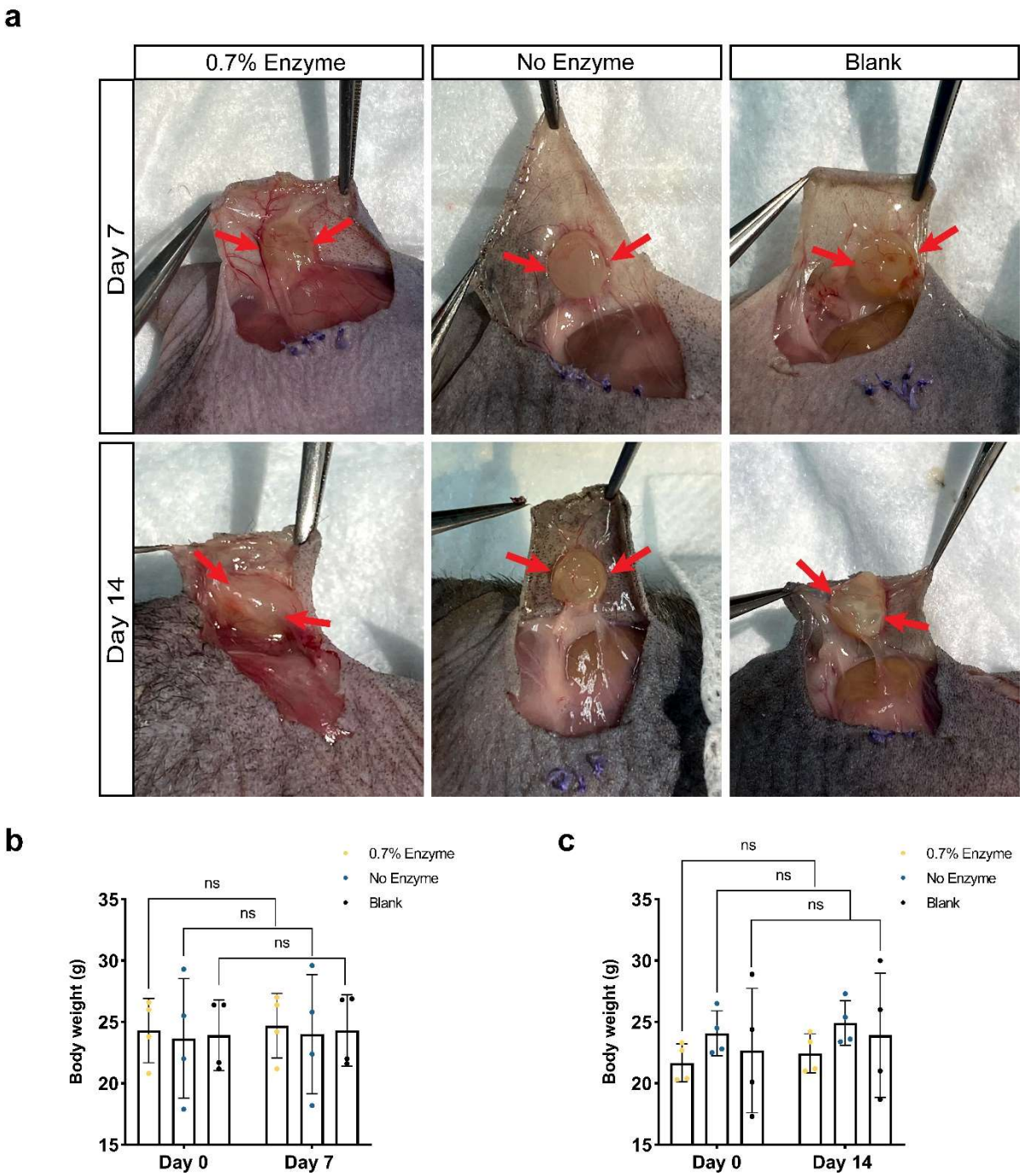


**Figure S7.** Experimental evaluation of subcutaneous implantation of hydrogels scaffolds in nude mice. a. Explanation of subcutaneously implanted hydrogels (denoted by red arrows) after 1 week and 2 weeks. Body weights (grams) in nude mice on day 0 (implantation) and day of sacrifice in all the three groups of hydrogels b. after 7 days c. after 14 days. NS indicates no significant difference.

**Table S2**. Average score of local irritation reaction to subcutaneous implants for day7

| Cell type/ tissue response | 0.7% Enzyme | No Enzyme | Blank | Control |
|---|---|---|---|---|
| **polymorphonuclear cells (PMN)** | 3.00 | 3.08 | 3.08 | 2.09 |
| **Lymphocytes** | 0.83 | 0.50 | 1.17 | 0 |
| **Plasma cells** | 0.25 | 0.08 | 0.33 | 0.09 |
| **Macrophages** | 2.83 | 2.42 | 2.67 | 1.27 |
| **Giant cells** | 0.17 | 0.00 | 0.00 | 0 |
| **Necrosis** | 0.42 | 0.67 | 0.67 | 0 |
| **A: subtotal inflammation score (×2)** | 15.00 | 13.50 | 15.83 | 6.90 |
| **Neovascularization** | 1.92 | 2.58 | 2.83 | 1.73 |
| **Fibrosis** | 1.83 | 2.00 | 1.58 | 1.64 |
| **Fatty infiltrate** | 0.08 | 0.08 | 0.17 | 0.27 |
| **B: Tissue response subtotal** | 3.83 | 4.67 | 4.58 | 3.64 |
| **Total (A+B)** | 18.83 | 18.16 | 20.41 | 10.54 |

Mean histopathological score of reaction based on the number of cells per high-powered field at 400x: 0 = 0 cells;1= 1-5 cells; 2 = 5-10 cells; 3 = heavy infiltrate; 4 = packed.

**Table S3**. Average score of local irritation reaction to subcutaneous implants for day 14

| Cell type/ tissue response | 0.7% Enzyme | No Enzyme | Blank | Control |
|---|---|---|---|---|
| polymorphonuclear cells (PMN) | 3.08 | 2.08 | 2.75 | 2.91 |
| Lymphocytes | 0.00 | 0.00 | 0.00 | 0.00 |
| Plasma cells | 0.00 | 0.00 | 0.00 | 0.00 |
| Macrophages | 3.00 | 3.00 | 2.83 | 1.82 |
| Giant cells | 0.08 | 0.00 | 0.08 | 0.00 |
| Necrosis | 0.33 | 0.25 | 0.92 | 0.45 |
| A: Subtotal inflammation score (×2) | 13.00 | 10.67 | 13.17 | 10.36 |
| Neovascularization | 2.75 | 2.50 | 2.00 | 0.91 |
| Fibrosis | 1.83 | 1.08 | 1.25 | 1.73 |
| Fatty infiltrate | 0.00 | 0.00 | 0.00 | 0.00 |
| B: Tissue response subtotal | 4.58 | 3.58 | 3.25 | 2.64 |
| Total (A+B) | 17.58 | 14.25 | 16.42 | 13.00 |

Mean histopathological score of reaction based on the number of cells per high-powered field at 400x: 0 = 0 cells;1= 1-5 cells; 2 = 5-10 cells; 3 = heavy infiltrate; 4 = packed.