# Shape Changing Photodegradable Hydrogels for 2D to 3D Cell Culture

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**Statement of Purpose:** Standard practice for studying cell biology *in vitro* is on rigid 2D substrates. While 2D culture is often higher throughput, and has more standardized techniques than 3D approaches, it does not allow the complexity with which to recapitulate the form and function of complex organs. However, if we look to developmental biology, we find that even the most complex biological structures are derived from fairly simple units that undergo numerous shape transformations. For example, the vertebrate central nervous system is derived during neurulation when a mass of ectodermal cells, termed the neural plate, folds and undergoes shape transformations to form the neural tube—eventually forming the brain and spinal cord. Developing scaffolds that mimic the time sensitive shape change processes of morphogenesis has important implications for developmental biology and tissue engineering. Here, we demonstrate self-folding photodegradable hydrogels that permit cell seeding, or encapsulation, in initially 2D, or flat, films. A light stimulus is used to induce degradation of photolabile crosslinks in swollen hydrogels. Upon re-equilibration, heterogeneities in the hydrogel swelling lead to 3D folding in the presence of cells.

**Methods:** Photodegradable films containing *ortho*-nitrobenzyl (*o*NB) moieties were crosslinked by radical polymerization. At user-defined time points, long wave UV light (365 nm) was used to generate heterogeneities in crosslink density causing differential swelling. Because light is absorbed and thus attenuated through the depth of the film, heterogeneities in crosslink density were introduced by exposing the gel to UV light on one side. Additionally, photomasks were used to generate more complex patterns along the lateral plane of the gel. Macroscopic folding was imaged using a digital camera. Mouse myoblast, C2C12 cells were used as a model cell line to study viability, proliferation and reorganization on the dynamic substrates.

**Results:** Using (365 nm) UV light, we generated curled, tubular structures after photodegrading one side of the 4 mm diameter, 150 μm thick planar films. The maximum cross-sectional width of the folded structures ranged from 1 to 4 mm. Spirals and containers were also generated using photomasks to pattern along the films' x-y direction (Figure 1). Tuning the ratio of photolabile *o*NB to non-photolabile polyethylene glycol (PEG) diacrylate crosslinks (2:1, 4:3, 1:1 or 3:4 molar ratio) permitted modulation of the tubular structure curvatures. We demonstrated that higher *o*NB concentrations led to more folding, such that the 2:1 system curled the most and the 3:4 curled the least. Furthermore, by adjusting the light irradiance from 20-150 mW/cm$^2$ with a 600 s exposure time, folding was tuned in the 2:1 system. For exposures at less than 40 mW/cm$^2$, we did not observe tubes. Between 40-150 mW/cm$^2$, tubes with diameters of about 1.4 mm were formed. However, beyond 120 mW/cm$^2$, we observed damage to the gels—attributed to erosion.

The PEG based system was functionalized with an RGD peptide to support cell adhesion. C2C12s were seeded on gels and exposed to UV light on the opposite side at 1 or 3 days after seeding. Samples exposed 24 h after seeding were approximately 50% confluent. We observed a temporary change in morphology from elongated to more rounded at 2 days after folding. However, by day 6, the cells had regained their elongated morphology and occupied 80-90% of the inner surface of the folded tubular structures. Samples exposed 72 h after seeding were about 90% confluent at the time of exposure. In these samples, we did not observe a marked change in morphology, nor did the cells appear to be adversely affected by the folding as they continued to spread along the inner wall of the hydrogels.

In an effort to create a scaffold that can be remodeled by the cells, we also co-polymerized the photodegradable *o*NB macromer with methacrylamide functionalized gelatin (gelMA). By tuning the ratio of gelMA to *o*NB, we tuned the rate to maximum curling from minutes to days and generated tubular structures of less than 1 mm diameter.

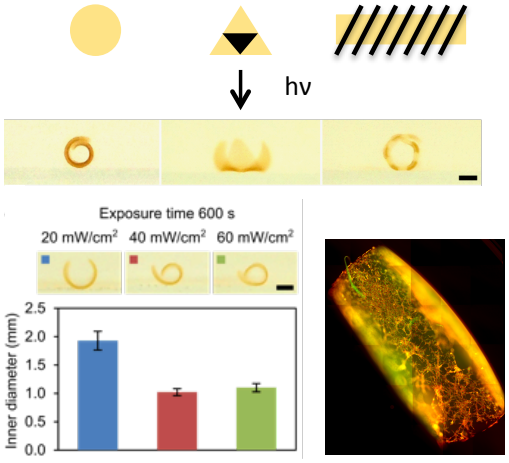


Figure 1. Top is a schematic of the photodegradable self-folding concept with and without photomasks and the resulting 3D shapes. Bottom demonstrates fluorescently labeled cells spreading along the inner wall of a folded gel and diameters of gels folded in the presence of seeded cells. Scale bars are 1 mm.

**Conclusions:** We demonstrate dynamic substrates for cell culture. Importantly, this is first biomaterial with user directed self-folding at a time point of choice, allowing 2D–3D shape transformation in the presence of cells.

**References:** (Käpylä, E.*; Delgado, S.M*.; Kasko, A. M. ACS Appl. Mater. Interfaces. 2016;*8* (28): 17885-17893.)
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