At first sight, these observations might seem alarming, but a number of points need to be considered. First, no similar observations were made with silk that was degraded using α-chymotrypsin to yield insoluble silk crystals and soluble hydrophilic domains; these showed no cytotoxicity at the maximum tested concentration ($IC_{50} > 225$ μg/ml), in sharp contrast to protease XIV degradation that generated soluble fragments with β-sheet structures (Numata et al. 2010). Second, protease XIV is a non-mammalian enzyme; therefore, its degradation products are not necessarily encountered *in vivo*. Third, the degradation products generated by chymotrypsin, a mammalian enzyme, showed no cytotoxicity. Fourth, *in vivo* studies, and indeed observations in human clinical trials and during routine use of silk sutures, have not shown any overt adverse effects due to inadequate biodegradation. The US Pharmacopeia classifies silk sutures as non-resorbable. However, this is based on the definition that the material "loses most of its tensile strength within 60 days" post-implantation *in vivo*. Silk sutures significantly degrade within 1 year, and they are completely resorbed within 2 years (Altman et al. 2003).

In vitro studies and mapping of the silk primary sequence to a known protease cleavage site indicated that serine proteases (e.g., α-chymotrypsin, collagenase) and matrix metalloproteinases (MMPs) (MMP-1, interstitial collagenase, and MMP-2, gelatinase A) are particularly active in silk degradation (Brown et al. 2015). Our current understanding of silk degradation (Brown et al. 2015) and emerging evidence suggest that the amorphous regions of silk hydrogels are degraded first. Thus, at an equivalent silk and β-sheet content, silk in hydrogel form is likely to be degraded fastest due to the open hydrogel frame structure, followed by porous scaffolds and films, where the monolithic structure restricts water ingress and thus hinders access of enzymes to the amorphous silk segments.

Silk Hydrogel Manufacture

Silk hydrogels can be broadly classified into physically (Ayub et al. 1993) and chemically crosslinked systems (Min et al. 1998).

Physically crosslinked silk hydrogels

A number of strategies have been explored to generate physically crosslinked silk hydrogels, including (i) ultrasound (Wang et al. 2008), (ii) vortexing (Yucel et al. 2009), (iii) CO_2 acidification (Floren et al. 2012), (iv) non-solvent induced phase separation (Kasoju et al. 2016), (v) electrical fields (Leisk et al. 2010; Lu et al. 2011), (vi) temperature (Kim et al. 2004), (vii) osmotic stress (Kim et al. 2004; Ribeiro et al. 2014) and (viii) pH (Ayoub et al. 2007). The underlying basis of all these systems is the self-assembling behaviour of silk, which forms hydrogels due to the physical entanglements and hydrogen bonding between hydrophobic domains of the silk block copolymer. Under aqueous conditions, this self-assembly into micelles is a thermodynamic process, whose kinetics depend on molecular mobility, charge, hydrophilic interactions and concentration (Lu et al. 2012). Therefore, any changes in these parameters due to the chosen processing technique will directly affect the final format of the silk.