cells within the hydrogel network. However, this silk-induced inflammatory response was less intense than that observed for collagen hydrogels, which attracted numerous neutrophils, eosinophils and macrophages that infiltrated the material and subsequently degraded the hydrogel completely within 4 weeks. At week 4, the inflammation around the silk hydrogel was greatly reduced and the hydrogel had cracks that were populated by spindle shaped cells; at 3 months, no inflammatory cells could be detected in or around the silk hydrogels, but vascularisation was apparent and the interstitial spaces were populated by stromal cells. Corroborating these observations are results from subcutaneously implanted 8% w/v silk hydrogels in rats, where significant hydrogel remodelling was initiated at 15 weeks post implantation, resulting in vascularisation, loss of hydrogel shape and degradation (Hamilton et al. 2015).

Silk Biodegradation

Most studies reporting the *in vivo* biodegradation of silk have assessed films and scaffolds; only a few studies have characterized silk hydrogels. One study used sonication-induced sol-gels that were preformed *ex vivo* and subsequently surgically implanted in mice. Cylindrically shaped 8 × 6 mm silk hydrogels generated from a 4% w/v silk solution were subcutaneously implanted in nude mice and the biological response assessed over 12 weeks (Etienne et al. 2009). At the endpoint of this study, fragmentation of the silk hydrogel was reported, although the hydrogel maintained its shape and showed signs of vascularisation (Etienne et al. 2009). However, the exact extent of silk hydrogel degradation was not assessed as well as the process of vascularisation.

Silk is biodegradable due to its susceptibility to proteases and enzyme-catalysed hydrolysis reactions. In particular, the disulphide bond between the light and heavy chains, as well as the amorphous silk sequences, are highly susceptible to degradation. In contrast, the crystalline regions are most resistant to proteolytic degradation due to reduced chain flexibility and access. Therefore, the β-sheet content has a protective effect on silk degradation both *in vitro* (Li et al. 2003; Horan et al. 2005; Meinel et al. 2005; Numata et al. 2010; Brown et al. 2015) and *in vivo* (Meinel et al. 2005; Wang et al. 2008). However, differences in packing geometries exist within the crystalline regions, ranging from tight to looser chain packing. This, in turn, results in differences in degradation behaviour; the tightly packed crystalline regions are made up of highly ordered β-sheets while the looser packing contains less ordered β-sheets as well as turn-and random-coil structures (Numata et al. 2010). In particular, the more loosely packed crystalline regions are susceptible to degradation and are degraded first.

Protease XIV is a useful proteolytic model enzyme for uncovering some of the fundamentals of silk degradation over short study intervals (e.g., hours to days). For example, protease XIV studies showed that digestion of the more loosely packed β-sheets yielded nanofibrils around 4 nm thick and 80–100 nm wide that persisted over the course of the *in vitro* degradation study (24 h), as well as soluble silk fragments. Cytotoxicity studies indicated that these protease XIV digested silk samples reduced cell viability (IC_{50} 75 μg/ml), whereas disease-associated β-sheets of amyloid β-peptide fibrils (IC_{50} 20 μg/ml) were more cytotoxic (Numata et al. 2010).