

the rat MSCs that were isolated from bone marrow and fibronectin [35]. Microdrops of low viscosity alginate were applied on the super hydrophobic surface, and then CaCl_2 was added on top of the hydrophobic surface and allowed for cross-linking. Over the conventional technique, the super hydrophobic surface method is advantageous in tissue engineering in reducing mechanical forces and aggregation of particles, and has also shown excellent permissibility for nutrients and oxygen, although super hydrophobic surface-based alginate gels administered optimum survivability of cell during entrapment for several days [35].

10.3.4 Internal Gelation

Internal gelation process is a process not much explored like external gelation, but in the future, there will be chances of increasing the use of this process because of direct site injection. Internal gelation is an *in situ* hydrogel formation process. By internal gelation, the alginate hydrogels are formed at the site of interest by using polymeric solution with a combination of cells [36–40].

The most acceptable approach in the internal gelation is gradual gelation with low solubility using divalent cation salts that slow down gelation rate with time. Most commonly used divalent cations are calcium carbonate (CaCO_3) and calcium sulfate (CaSO_4). Both of these divalent cations have low solubility in pure water at neutral pH, but may be soluble under acidic conditions. This low solubility allows uniform distribution in the alginate solution [30, 41]. Later on, at acidic pH, free calcium ions can be released with glucone-d-lactone (GDL). To attain a neutral pH, the molar ratio of CaCO_3 , the CaCO_3/GDL molar ratio, can be set [30, 36]. A photoacid generator (PAG) is an alternative to GDL that also helps to release out free Ca^{2+} and H^+ [42]. The use of Ca^{2+} chelators also advocates photoactivated internal gelation, which was mixed with alginate solutions and exposed to light that decreases the affinity to Ca^{2+} in an irreversible manner [43]. Internal gelation method is most applicable in improvements in mechanical properties and homogeneity.

10.3.5 Covalent Cross-Linking

Alginate hydrogels are produced through a various methods; out of these methods, covalent cross-linking provides a more mechanically stable and stronger gel than other linking methods. Once a material is covalently linked, it no longer meets the injectability criteria [44, 45]. PEG-diamine molecules with different molecular weights are used as a covalent