# Mesenchymal Stem Cell Pellet Delivery Platform for Functional Repair of Cartilage

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**Introduction:** High-density seeding of mesenchymal stem cells (MSCs) as pellets is an effective way to initiate chondrogenesis via mesenchymal condensation [1]. However, pellet condensates have to be cultured for weeks in bioreactors [1] and the resulting tissue may lack mechanical robustness. It is also challenging to provide cell pellets with a form that is matching the size and shape of the defect. Seeding the cells to the scaffolds may provide with the mechanical support and form; however, these approaches lack mesenchymal condensation and chondrogenesis may not be attained reliably. Therefore, synergizing the merits of mesenchymal condensation and the mechanical framework of scaffolds may result in functional cartilage repair. We are proposing an innovative woven collagen fabric with macroporous channels that can accommodate cell-pellets.

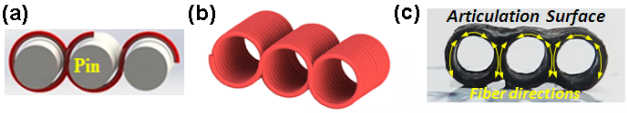


**Figure 1**. Fabrication of scaffold by weaving electrochemically aligned collagen threads over an array of pins (a), schematic of scaffold following the recover from pins (b), and an actual scaffold with molecular alignment in threads mimicking the native cartilage's arcade feature (c).

**Materials and Methods:** Collagen threads were fabricated by an automated electrochemical alignment device [2]. The threads were woven with the aid of evenly spaced pins (Fig. 1a-c). Woven structure was fixed with the help of two electrocompacted collagen sheets attached on top and bottom faces of woven scaffold. The assembly was crosslinked in genipin and recovered from the pins, leaving cylindrical pore regions (1.5 mm diameter) within which cells pellets were placed. Three million human bone marrow derived MSCs at passage 3 were cultured in pellet form for 3 days and then transferred to the scaffolds holes. Free-standing pellets were also cultured as positive controls. Collagen and GAG contents were measured biochemically. Compression tests (1 mm/min) were performed on empty scaffolds and scaffolds filled with cell pellets. Scaffolds filled with cells were cultured for 4 weeks (Fig. 1d), fixed and processed for immunohistology. Weights of scaffolds with pellets and free-standing pellets were measured.

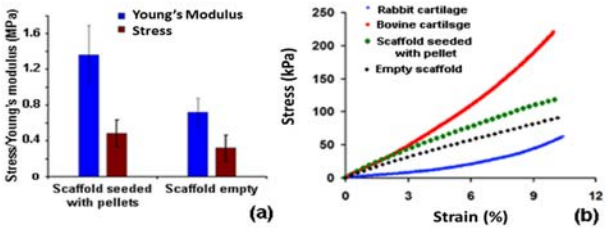


**Figure 2**. Compressive stress and Young's modulus of empty and pellet seeded scaffold (a), a comparison between scaffold compressive behavior with animal cartilage (b).

**Results:** The stiffness of the woven arcade structure –absent with cells- was intermediate between rabbit cartilage and bovine cartilage (Fig 2b). Cell-seeded scaffolds had significantly greater stiffness and compressive stress values than those of the blank scaffolds (Fig. 2a). Histological sections showed that cells had round chondrocyte like morphology at the core of the cylindrical pores (Fig. 3a) which were positive for GAG production by safranin-O and type II collagen by immunohistochemistry (Fig. 3b & c), indicating chondrogenic differentiation of the MSCs within the confines of scaffolds. The amount of GAG's and collagen made by cells in scaffold were comparable to those made by free-standing pellets, demonstrating that the scaffolds do not have any negative effect on cell biological activities and matrix production.

**Conclusions:** To the best of our knowledge this is the first time MSC pellets are accommodated within a scaffold network. These results illustrate that the woven collagen scaffold is a mechanically competent vehicle to deliver MSCs pellets. Remarkably, the scaffold was able to match cartilage stiffness at a high pore volume of 80%, likely due to biomimicking of the arcade architecture. Such baseline stiffness would be conducive to shorter culture times and earlier implantation in future in vivo applications.

[1] Bhumiratana, S., et al., Proc Natl Acad Sci U S A, 2014. 111(19): p. 6940-5.

[2] Mousa Younesi AI, Vipuil Kishore, James M. Anderson, Ozan Akkus.. Advanced Functional Materials. 2014.

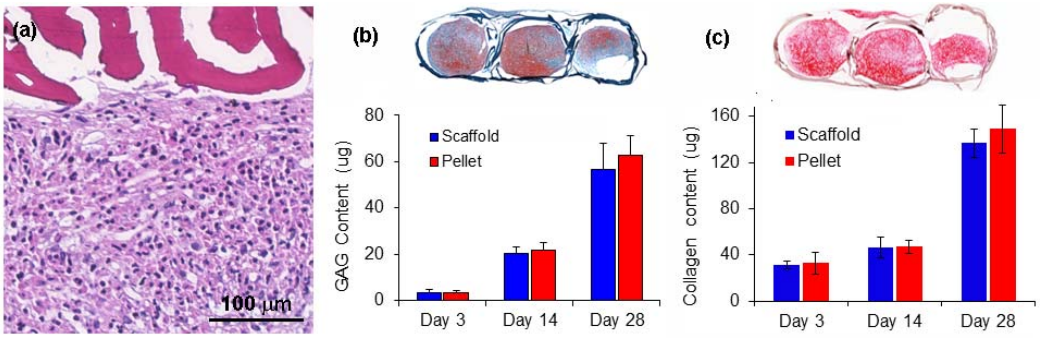


**Figure3.** H&E staining demonstrates round cell morphology in scaffold (a), qualitative (histology and immunostaining) and quantitative analysis of increase in GAG's (b) and collagen (c) productions by cells in scaffold.