Rapid Production of a Tubular Dense Collagen Construct for Tissue Engineering

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Statement of Purpose: Due to the intrinsic complexity of tissues and organs, only planar tissues have achieved clinical applications in tissue engineering. In contrast, tissue engineered tubular constructs present a complex geometry and architecture, limiting the accomplishment of suitable substitutes. Type I collagen is an optimal candidate for scaffolding due to the high biocompatibility and processability. However, due to their highly-hydrated nature, collagen gels exhibit unstable geometrical and low mechanical properties, and are prone to cell-mediated contraction, thus often requiring additional modifications. Plastic compression (PC) technique rapidly produces dense collagen constructs with fibrillar densities equivalent to native tissue (Brown R.A. et al., Adv Funct Mater 2005; 15(11): p. 1762 1770). In this study, by application of PC a tubular dense collagen construct (TDCC) was developed and characterized. Moreover the effect of different collagen densities and diverse collagen sources were investigated.

Methods: Collagen gels were prepared using type I collagen in acidic solution from rat-tail tendon (RT, First Link, Brimingham, UK) and bovine dermis (BD, Devro Medical, San Jose, CA) at concentration of 2.05 mg/ml and 4.8 mg/ml, respectively. The collagen solution was cast in a rectangular mold (18x40x7 mm^3). After gelling, dense collagen scaffolds were produced by applying an unconfined compression of 0.8 kPa for 5 min on highly hydrated collagen gels. TDCC was then created by rolling a dense collagen sheet into three concentric layers around a cylindrical 3.8 mm polytetrafluoroethylene mandrel (Figure 1).

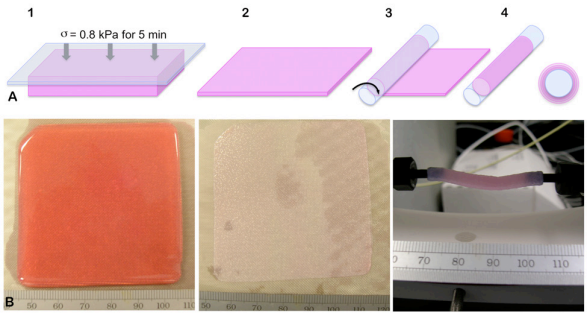

Figure 1.A. Schematic diagram of TDCC assembly. B. Gross view of as prepared collagen gel, dense collagen sheet post PC and TDCC.

The collagen fibrillar density (CFD) from RT and BD was measured by gravimetrical analysis. TDCCs were morphologically characterized with micro-computed tomography (microCT), scanning electron (SEM) and atomic force microscopy (AFM). Fourier transform infrared spectroscopy (FTIR) was used to characterize any potential differences in the chemical structure between collagen sources. In addition uniaxial and circumferential tensile testing, suture retention testing were carried out and an estimated burst pressure was calculated using Laplace's law (Nieponice A. et al., Biomaterials 2008; 29: p. 825–33).

Results: The PC efficacy was found transferable between collagen sources with a relative CFD increase of approximately 20 fold, leading to a final CFD of 14.12±0.56 wt% and 8.17±0.82 wt% for BD and RT, respectively. SEM micrographs showed an increased fibril bundle dimension in TDCC from BD compared to RT. The same result was confirmed by AFM analysis, where the average fibril diameter was significantly higher ($p<0.05$) in BD (0.326±0.04 μm) derivation compared to RT (0.133±0.02 μm). Some differences were detected in the chemical structure of the two proteins, as the higher resonance Amide I of RT indicates a more random organization compared to BD. In addition, the higher crystallinity index of BD was demonstrated to be a concentration-dependent factor rather than function of collagen source. MicroCT analysis was used to measure TDCC wall thickness. Uniaxial tensile test demonstrated mechanical superiority of BD compared to RT ($p<0.05$), reflecting the significant increase in CFD, fibril dimension and crystallinity index. Furthermore, the variation of ultimate tensile strength and apparent modulus has been shown to be directly proportional to the protein concentration, as for the crystallinity index. Circumferential tensile tests confirmed the different response of BD and RT ($p<0.05$), yielding ultimate circumferential tensile strength of 0.72±0.07 and 0.53±0.09 MPa, respectively, and apparent modulus of 2.85±0.44 and 1.3±0.23 MPa, respectively, both of which were one order of magnitude greater than values previously described for collagen-based tubular constructs (Nerem R.M. et al., Annu Rev Biomed Eng 2001; 3: p. 225-243). From the ring test, a theoretical burst pressure was calculated to be 1574±188 and 1223±153 mmHg for BD and RT, respectively. Suture retention strengths were measured at 151.22±11.25 and 116.69±18.23 grams-force for BD and RT samples, respectively and were comparable to native mammary artery and saphenous vein.

Conclusions: PC produces dense collagenous scaffolds with controllable mesoscale properties in less than an hour. TDCC from BD demonstrated a higher mechanical strength, in both uniaxial and circumferential directions, compared to RT derivation. Furthermore, TDCC revealed outstanding mechanical properties clearly superior to collagen-based approaches previously published. In addition a linear correlation between collagen protein concentration and chemical and mechanical properties was demonstrated. The tunable morphological properties, the excellent mechanical strength together with adequate suturability, present the TDCC as an optimum candidate for tubular tissue substitute.

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