Electronic Supplementary Material (ESI) for Biomaterials Science.

Development Of Wound Healing Scaffolds Capable Of Precisely-Triggered Release Of Nanoparticles

Supplementary Information

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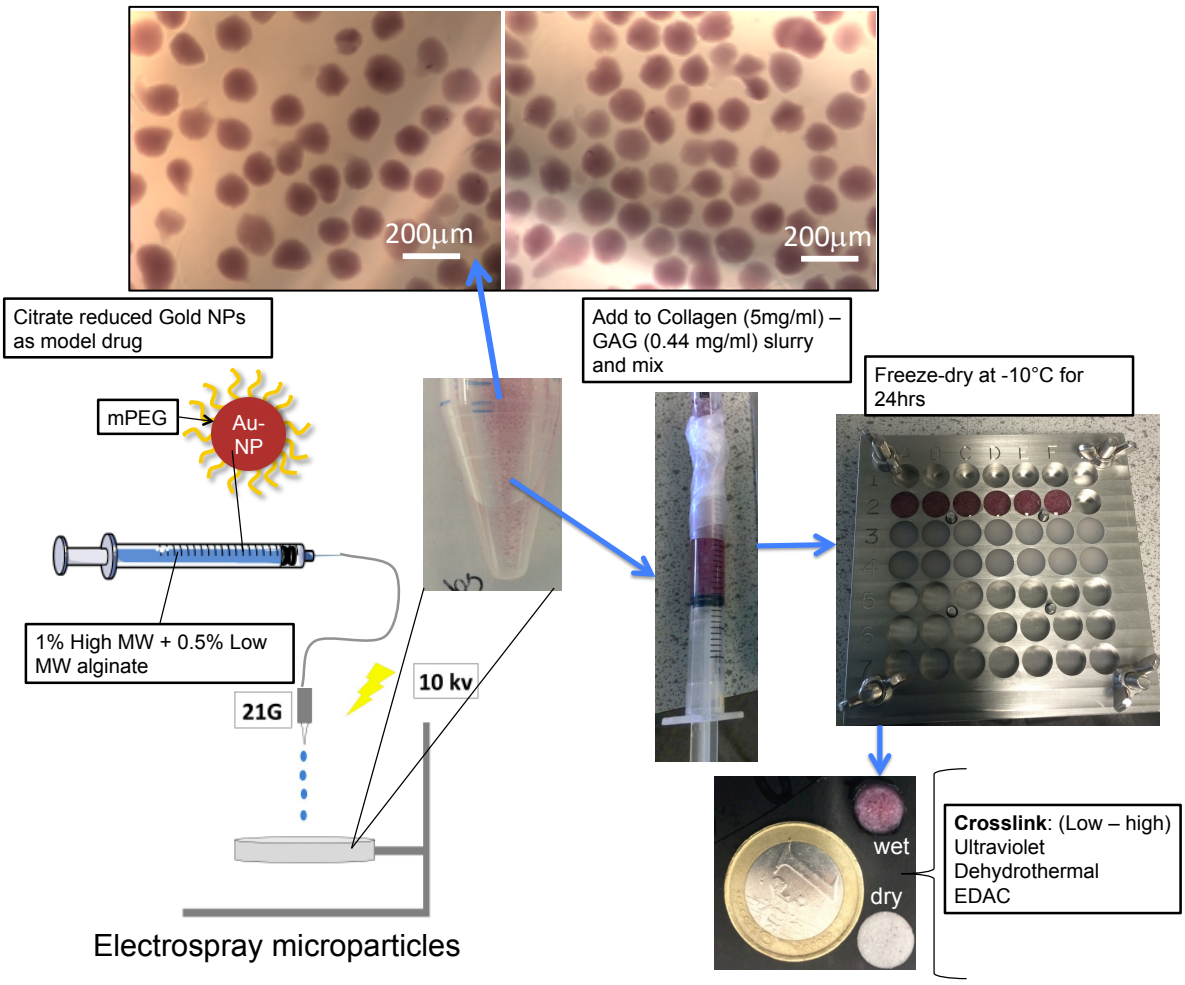
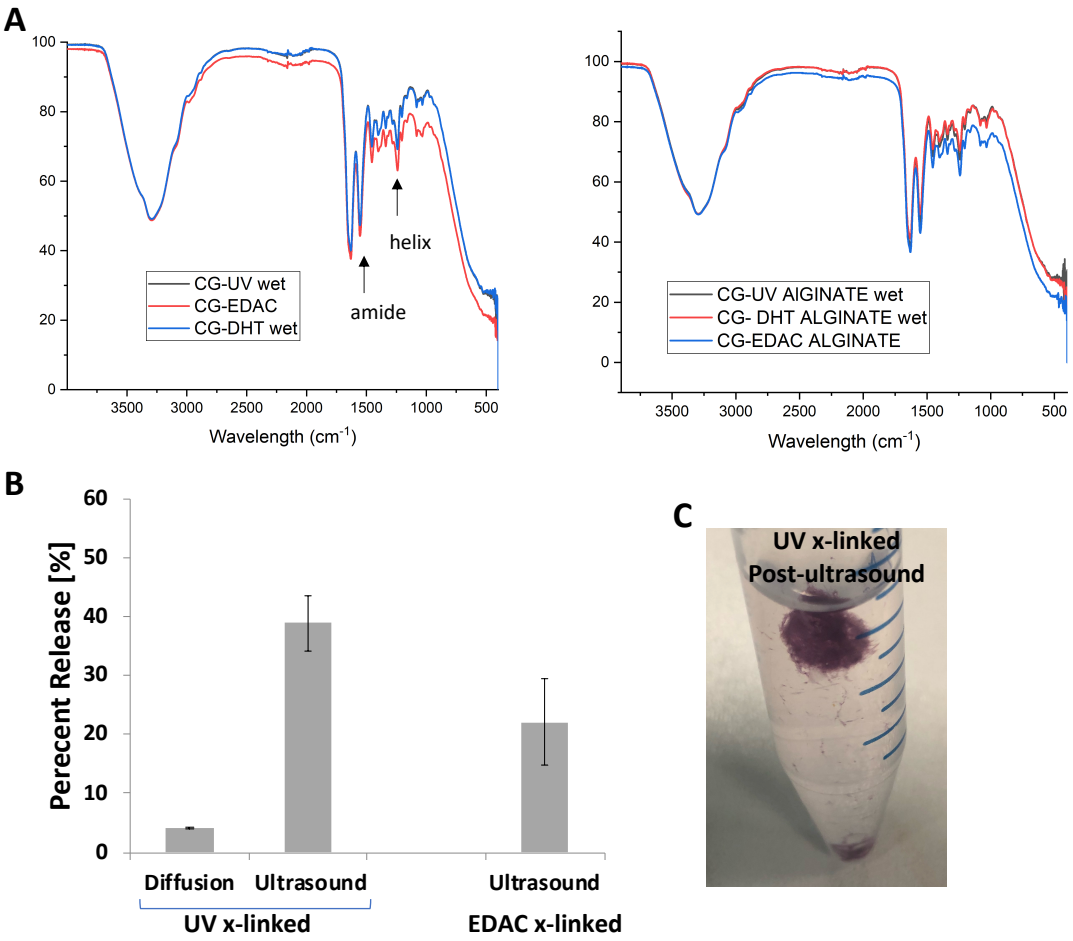


Supplementary Figure 1: Workflow for fabrication of microparticle-loaded CG scaffolds. Following gold nanoparticle and alginate mixing, microparticles are created by electrospraying (see inset: approximately 100µm diameter). These microparticles are directly mixed in collagen-GAG slurry using a luer-lock connector and cast in freeze drying trays. Freeze-drying is used to create a microporous scaffold. Crosslinking is performed using ultraviolet, dehydrothermal crosslinking and EDAC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) techniques.

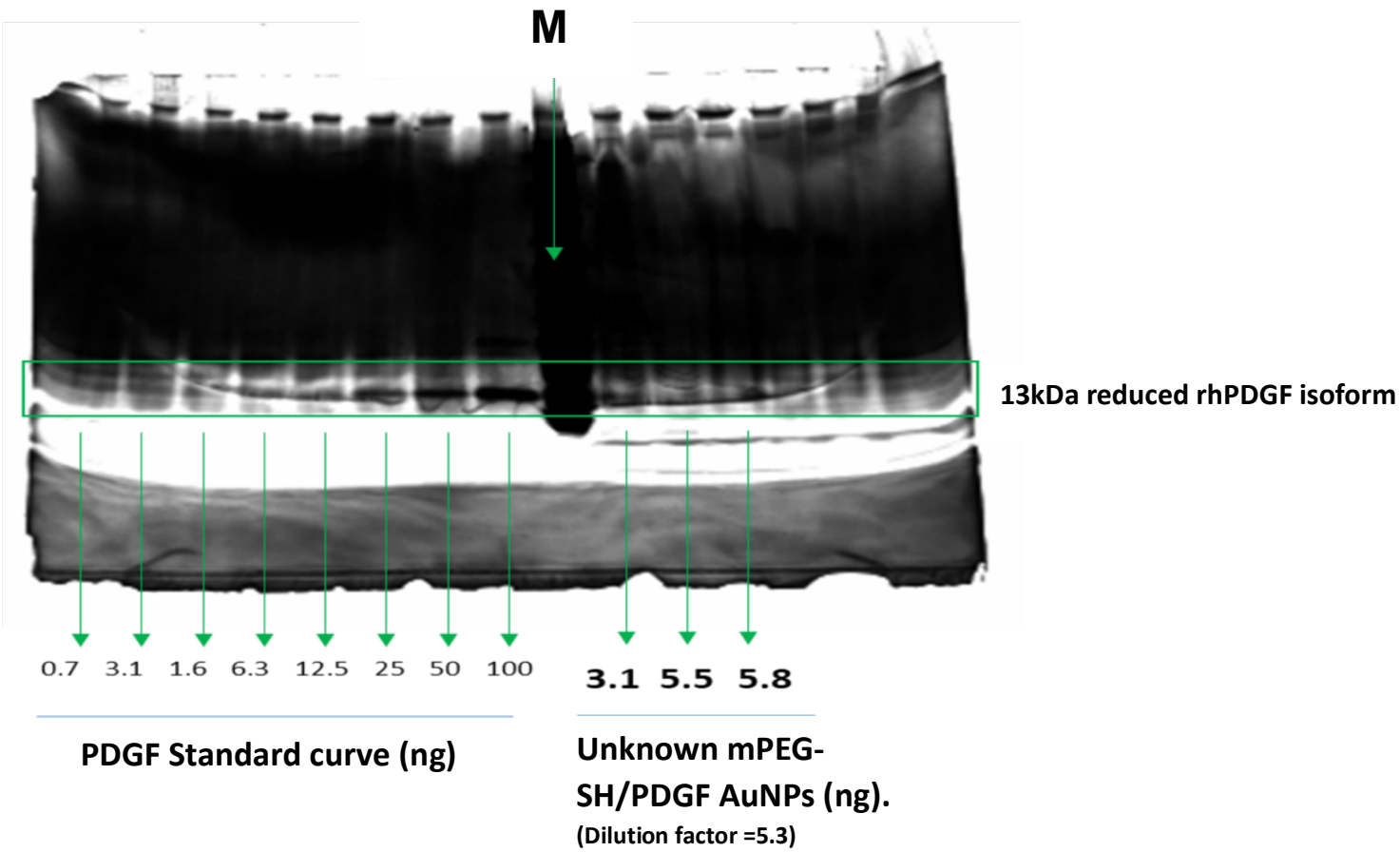


Supplementary Figure 2: (A) FTIR spectra of collagen undergoing crosslinking by UV, DHT and EDAC in the presence (right) and absence (left) of alginate microparticles. Signature bands for the collagen triple helix structure and the amide bonds formed by crosslinking are highlighted. (B) Release profiles for gold nanoparticles directly incorporated into collagen-based scaffolds, without alginate. For UV-crosslinked scaffolds, diffusion was very low, even when left for a several days, suggesting that breakdown of the collagen may be required for release to occur. In response to ultrasound (25% amplitude, 3 x 5 min cycles), 40% of the nanoparticles are released. The scaffold broke down macroscopically, however (see C). Interestingly, EDAC crosslinked scaffolds also demonstrated a release in response to ultrasound, however, no macroscopic damage was observed.

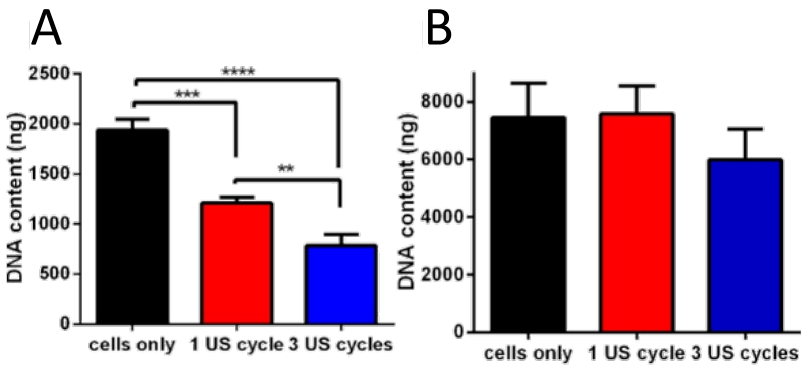
Supplementary Info 3: PDGF quantification on alginate using silver stain

Gold nanoparticles conjugated with PDGF were produced by standard methods as described earlier and used fresh for analysis. Following washing steps a sample of 40uL (AuNP-PDGE) was obtained for analysis from three different conjugations. The PDGF attached to the nanoparticles was processed by SDS-PAGE followed by silver staining. From each batch 7.5uL was mixed in Laemmli and loaded on a Mini-PROTEAN® TGX™ Precast Gels (Biorad # 4561096). A titration curve with a rhPDGF standard was run on the same gel. Following electrophoresis, the gel was quickly washed (5 min, RT, 1x) in ddH2O prior to fixation and staining. Silver staining was conducted according to manufacturer's instruction (Biorad, Silver Stain Plus Kit #1610449). Staining development was stopped when the 3ng band of the titration curve appeared (1 hour development). Gel images were acquired using a Amersham Gel and Blot imagind system (GE Healthcare). In order to facilitate the analysis contrast was enhanced to eliminate background. This was possible because standard curve and samples were on the same gel. Titration curve was generated and analysed using the Standard curve tool in Image Lab (Biorad). The unknown AuNP batches were interpolated on the curve to determine PDGF content on AuNP surface.

Gel analysis (Suppl. Figure 3) revealed that mPEG-SH/PDGF nanoparticles processed by SDS-PAGE/Silver staining had an association efficiency that ranged between 38-71%. Loaded initially with 43ng PDGF, AuNPs retained on average 25.4 ng on the surface (i.e. 59%). Supernatant and washes were also analysed, but PDGF was undetectable (below 0.1ng – data not shown) due to presence of confounding sugars (i.e. trehalose) which prevented concentration of volume.



Supplementary figure 3. Silver staining of mPEG-SH/PDGF AuNPs. PDGF conjugated AuNPs were produced by standard methods and analysed by SDS-PAGE and Silver staining. Left side: PDGF standard curve (range 0.7-100 ng); M: Marker lane; Right side: PDFG bound to mPEG-SH/PDGF AuNPs. Gel images were acquired with a Amersham GelDoc system and standard curve was generated and analysed using the standard curve tool in Image Lab (Biorad).

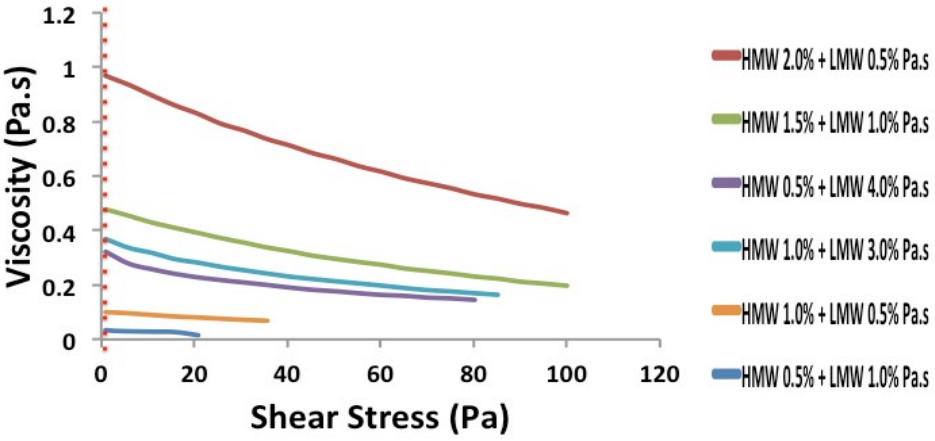


Supplementary Figure 4. (A) DNA content in collagen scaffolds where BJ cells were cultured for 3 days, and ultrasound was applied to the scaffolds homogeneously in a conical tube reveals a reduction in cell number (n=3, **p<0.01, ***p<0.001, ****p<0.0001 by Tukey's multiple comparison test). (B) By contrast, DNA content in pocketed scaffolds where BJ cells were cultured for 3 days and ultrasound was applied directly on pockets of scaffolds containing alginate hydrogels did not significantly reduce cell viability (n=3).

Supplementary Info 5: Development of injectable alginate for pocket filling

To create an alginate gel suitable for filling of the pockets, a range of high and low molecular weight alginate combinations were tested.

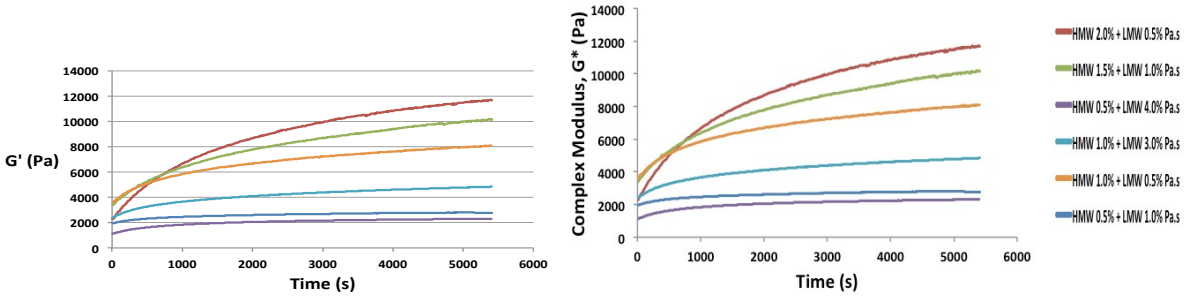
Pre-gel Viscosity:



Supplementary Figure 5a: Shear thinning curve of pre-gel solutions. The pre-gel viscosity was determined from the curves lowest point of stress.

The pre-gel viscosity of each gel with different biomodal MW distribution was investigated under increasing shear stress from 1 to 100Pa at a temperature of 25°C. The results of the pre-gel viscosity assessment of each hydrogel is shown above in Figure 5a. Increasing HMW concentration increased viscosity, whereas the pregel viscosity was a lot less sensitive to changes in LMW percentage.

Time Sweep of Crosslinked Hydrogels:

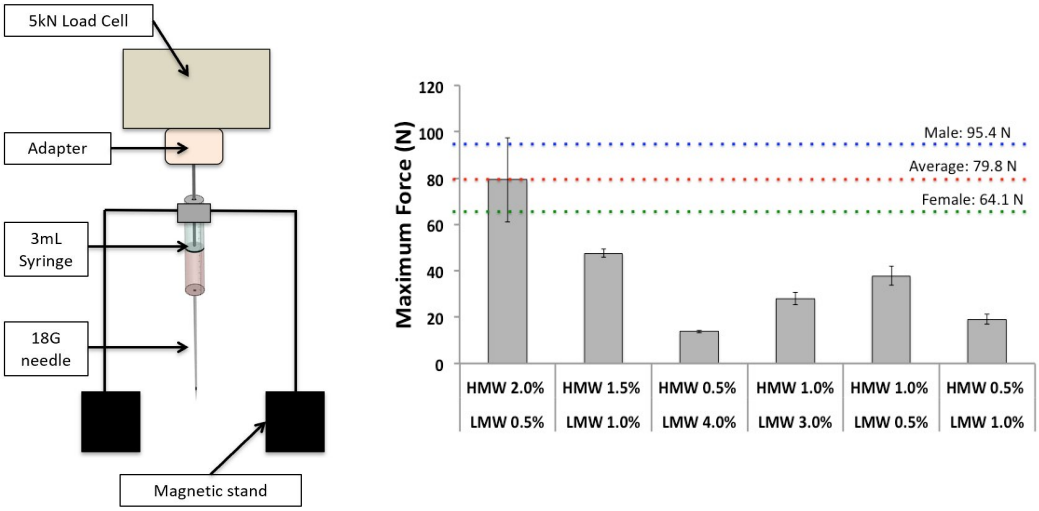


Supplementary Figure 5b: Rheological time sweep of crosslinked hydrogels, confirming gelation and comparing the elastic (G') and storage (G'') modulus

To investigate the gelation process of each hydrogel, gels were crosslinked and injected onto the testing plate within one minute of mixing. A time sweep was then performed at a constant oscillating stress of 1Pa at a constant temperature of 25°C for 90 minutes. A solvent trap was used to prevent evaporation of water from samples during the test. The results of the time sweep for each hydrogel is shown in Suppl Figure 5b. Gels with a larger HMW% had higher G'. Additionally, the hydrogels with a higher contribution of HMW alginate tended to take longer to achieve a plateau in G', which indicates that they are still undergoing crosslinking.

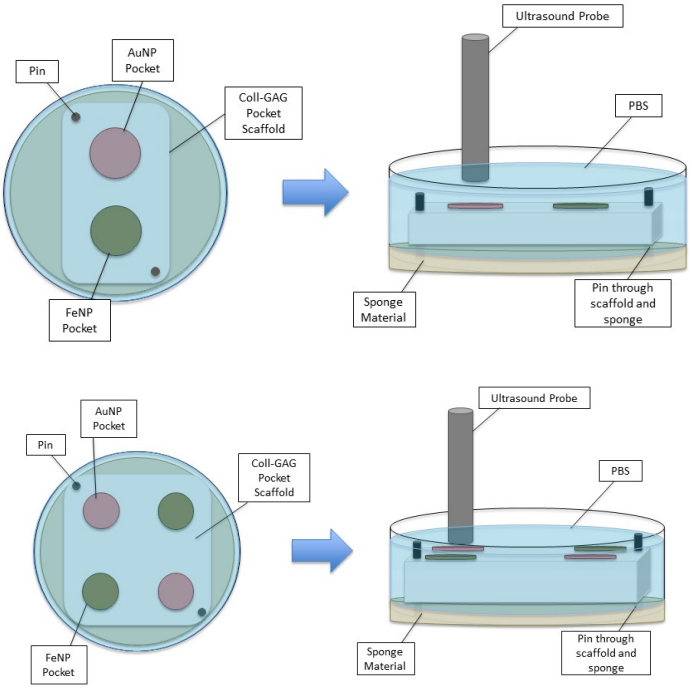
Injection Force Characterization:

Injections are most commonly performed with the thumb pushing the piston of the syringe while the index and middle finger are used to stabilise the syringe [1]. The average maximum force generated using this technique is 79.8N (Males 95.4N and Females 64.1N) [1] The force generated varies and it depends on the user's musculature and capability in generating force. Hence, it is important to ensure that the chosen injectable hydrogel is below this maximum injection force capability to ensure it is usable in a clinical setting. The maximum forces required to inject each hydrogel into air were assessed using a customised injection force rig on a Zwick Roell Z005 material testing system at room temperature using a 5kN load cell (see Supplemental Figure 5c). The maximum injection force was determined as the maximum force recorded during a 2.5mm displacement at a cycle speed of 0.21mm/s. Hydrogels were crosslinked and left in a cold room overnight prior to testing. The experiment was repeated six times for each hydrogel.



Supplemental Figure 5c: A customised injection force rig was used to make measurements of the maximum force required to inject hydrogels through a syringe (3mL Luer-Lock syringe, 18G needle). The average maximum injection force for males, females and both genders combined are depicted as the dotted blue, green and red lines respectively.

The results of measuring the maximum injection force for each hydrogel matches the G' trends and it also reflects that the hydrogels are within the injectable range as the maximum forces required to inject hydrogels are lower than the average maximum force generated during injection using a syringe needle system. Based on these considerations, and our experience with release from these alginate systems, the 1% HMW and 3% LMW gels were chosen as a suitable gel for the pocket scaffold studies.



Supplementary figure 6: Ultrasound release study setup for (A) Two Pocket Collagen-GAG Scaffold and (B) Four Pocket Collagen-GAG Scaffold

References

1. Vo, A., Doumit, M. & Rockwell, G. The Biomechanics and Optimization of the Needle-Syringe System for Injecting Triamcinolone Acetonide into Keloids. *J. Med. Eng.* **2016**, (2016).