Alginate Microsphere/Hyaluronic Acid Hydrogel Composites for Controlled Delivery of TGF-β3 to Enhance Encapsulated MSC Chondrogenesis

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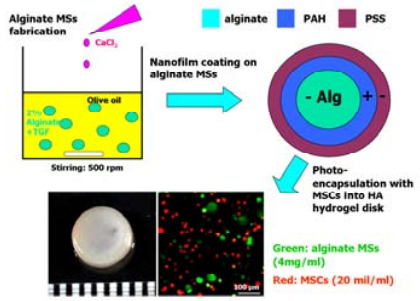
Statement of Purpose: Transforming growth factor-beta (TGF-β) superfamily members have been shown to be a key requirement for chondrogenesis of mesenchymal stem cells (MSCs)[1]. Additionally, one study demonstrated that continuous application of TGF-β inhibited hypertrophy of MSCs[2]. Therefore, successful tissue engineered cartilage implants using MSCs may require prolonged local delivery of TGF. Alginate microspheres (MSs) have been used widely for controlled delivery of growth factors due to advantages such as biocompatibility, high encapsulation efficiency, and mild fabrication conditions[3]. With these benefits in mind, the objective of this study was to develop an alginate-based delivery vehicle for TGF-β3 and test its efficacy in inducing human MSC chondrogenesis and neocartilage formation within hyaluronic acid (HA) hydrogels, which have been shown to be an effective scaffold material for chondrogenesis of MSCs in the presence of growth factors[4].



Group	Nanofilm coating	TGF encapsulated in gel	TGF supplemented in medium
Ca	*no*	*100ng TGF in MSs/disk*	-
Ca 1L	*yes*	*100ng TGF in MSs/disk*	-
Cont+T	-	*Blank MSs w/o TGF*	*10 ng/ml*
Cont- T	-	*Blank MSs w/o TGF*	-
T in gel	-	*100ng TGF /disk (w/o MSs)*	-

Figure 1 Fabrication, coating and photo-encapsulation of alginate microspheres with MSCs into HA hydrogels. Bar = 100 µm

Methods: Alginate MSs were prepared using an emulsion/external gelation protocol[5]. Using a method modified from Srivastava et al. alginate MSs were coated with a layer of self-assembled nanofilms consisting of poly(allylamine hydrochloride) (PAH, MW15 kDa) and poly(sodium 4-styrenesulfonate) (PSS,MW1 MDa) (Ca 1L)[6]. Alginate MSs were incubated in chondrogenic medium at 37 ˚C and the released TGF was quantified with an Elisa kit (R&D). Methacrylated HA (MeHA) was synthesized as previously reported[7]. Human MSCs (Lonza) were expanded to passage 3. MSCs and alginate MSs were photoencapsulated[4] in 1.5wt% HA hydrogel disks (Ø5mm, 2.5mm thickness) and cultured in chondrogenic media supplemented with or without TGF-β3 (10 ng/ml) (**Figure 1**). Young's moduli (E_Y) of samples were calculated from static unconfined compression testing and GAG and collagen content were determined with DMMB and hydroxyproline assays, respectively, as in[4]. Samples for histological analysis were fixed in 4% formalin for 24 h and embedded in paraffin (stained with Vectastain ABC kit and the DAB Substrate kit for peroxidase, Vector Labs). Statistical comparisons were performed using ANOVA and Tukey's HSD post hoc analyses (α=0.05).

Results: Nanofilm coating reduced the initial burst release of the TGFβ3 from alginate MSs in the first 2 days (Ca 1L vs. Ca, **Figure 2** A,B). Tissue engineered cartilage disks containing nanofilm coated MSs (Ca 1L) developed similar levels of mechanical stiffness, GAG and collagen content compared to the positive control (Cont+T), whereas these values for groups with uncoated MSs or direct TGFβ3 encapsulation without MSs were all lower than the Cont+T group (**Figure 2** C,D,E). The negative control group (Cont-T) showed minimal cartilage matrix production and decreasing cell viability, as indicated by viability staining (not shown) and DNA content in the absence of TGF-β3 (**Figure 2** F).

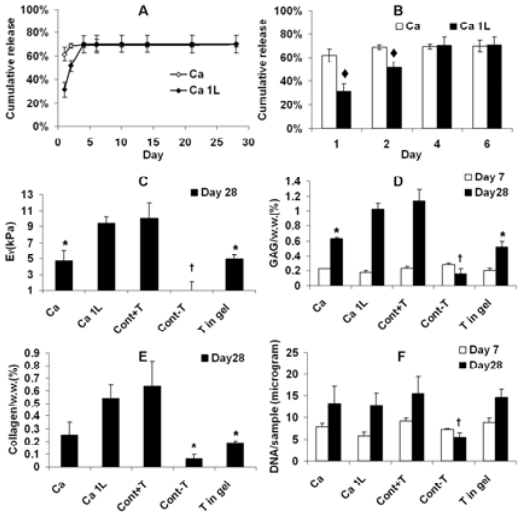


Figure 2 Release profiles of the TGFβ3 loaded alginate MSs (A B). Young's modulus (C), GAG (D), collagen (E) and DNA content (F) for all groups. ♦p<0.05 vs. Ca; *p<0.05 vs. Cont+T; †p<0.05 vs. all other groups at the same culture time (n=4)

Conclusions: Results from this study demonstrated that nanofilm coating delayed the initial burst of TGF-β3 from alginate MSs resulting in higher levels of cartilage specific matrix deposition by MSCs in HA hydrogels and superior mechanical stiffness compared to uncoated MSs and a bolus encapsulation of TGF-β3 over 28 days of in vitro culture. Ongoing studies are assessing the efficacy of this TGF delivery vehicle in vivo, as this approach may be needed for cartilage tissue engineering approaches were MSCs are directly implanted into defects.

References: [1]Johnstone B.Exp Cell Res 1998;238: 265-72 [2]Mueller MB.Arthritis Rheum 2008;58: 1377-88 [3]van de Weert M.Pharm Res 2000;17: 1159-67 [4]Chung C.Tissue Eng Part A 2009;15: 243-54 [5]Jay SM.J Control Release 2009;134: 26-34 [6]Srivastava R.Macromol Biosci 2005;5: 717-27 [7]Smeds KA.J Biomed Mater Res 2001;54: 115-21