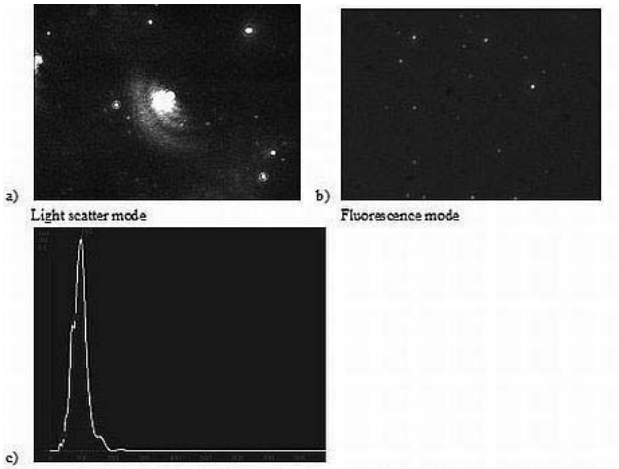

Figure 1. a) NTA video frame showing 100 nm fluorescent polystyrene beads in FBS under light scatter where ALL particles are seen, making measurement on the particles of interest difficult. b) NTA video frame of 100 nm polystyrene beads under fluorescent mode, where only the bead of interest is characterized. c) NTA concentration and size distribution data from video b.

Using NTA, sizing (10 nm to 1 µm) and concentration measurements of particles in various media are obtained. The particle-by-particle measurements lead to accurate size distributions for polydisperse, low concentration nanoparticle samples (10^8 particles/mL) commonly encountered in therapeutic and diagnostic applications. Through combinations of the measurements NTA offers, such as size, concentration, zeta potential, and fluorescence, NTA is a powerful, high resolution multi-parameter method with the capabilities to characterize and monitor particles in biological environments for toxicological and risk assessment studies.

265. Molecular and Cellular Analyses of Platelet-Rich Plasma Preparations

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Muscle injuries account for a large number of all injuries sustained by sports' participants. Despite, the treatment for these injuries has been the same for decades. One new treatment is cell therapy using PRP (platelet-rich plasma) but its use is still controversial and the results vary greatly. One of the reasons for this might be the large array of preparation methods. Our propose are compare different PRP preparations. Four milliliters of blood were collected from C57Bl/6 mice using a triangular lancet and placed in a glass tube containing sodium citrate 3.2% to prepare PRP based on the 5 most used methods wich showed in table 1. Cell quantification was performed by impedance methodology. To determine the concentration of growth factors (TGFß-1, HGF and PDGF-AB) by ELISA the PRP was frozen in liquid nitrogen for 1 minute and then rapidly thawed at 25°C; this process was repeated 3 times.

PRP separation methods

Speed (xg/min) 1st centrifugation	Speed (xg/min) 2nd centrifugation	Platelets are separated from BC	Protocols
200/20	400/10	No	1
400/10	1600/10	No	2
160/20	400/15	No	3
1125/10	1960/15	No	4A
1125/10	1960/15	Yes	4B
300/15	1000/10	Yes	5A
300/15	1000/10	No	5B

None of the protocols previously described to obtain PRP in mice distinguish between the actual PRP and the layer of white blood cells – the buffy coat (BC). The only exception is the protocol 5A and 4B in which the PRP is composed entirely by platelets. In order to obtain PRP with only platelets, we modified the protocols 1,2 and 3. In these preparations, the platelet counting fell drastically showing that the majority of the platelets were in the BC. Four protocols were used for additional analysis; 4A and 5B do not separate the BC from the PRP (PRPBC) while the 4B and the 5A make this separation. Table 2 shows the percentage of platelets and leukocytes in relation to the whole blood count and the concentration of the different growth factors measured.

Quantification of leukocytes and platelets in the PRP and growth factors dosage

	4A	4B	4B	5A	5A	5B
	PRPBC	PRP	BC	PRP	BC	PRPBC
P (%)	103.3± 1.11a	3.4±1.4	32±5.7	39.9± 10.2b	44.8±13.6	80.2±17a
L (%)	86.4±2.3	0	83.8±4.3	0.7±0.8	53.0±11.3	45.2±1.8
TGFB-1	21263± 2152a	11925±289	25283±1862	24152± 896b	36264±11687	67746±1207a
HGF	5747± 208a	210±0.7	7744±615	3098± 1557b	7700±989	4737±2012a

P: % platelets L: % leukocytes All values were expressed by average ± standard deviation. a: $p<0.05$ when PRPBC was compared with PRP b: $p<0.05$ when PRP-5A was compared with PRP-4B

There is a greater platelet recovery when BC was not separated from PRP. PRPBC-4A presented TGFß-1(2-fold), PDGF-AB(10-fold) and HGF(25-fold) more than PRP-4B. PRPBC-5B presented TGFß-1 (2-fold), PDGF-AB (3-fold) more than PRP-5A. The PRP-5A presented TGFß-1 (2-fold), PDGF-AB (6-fold) and HGF (15-fold) more than PRP-4B. These data indicate that there are large variations among PRP preparations and the PRP rich in platelets, growth factors and free of leukocytes can be obtained only by the 5A protocol. Variations of PRP quality are very concern because it can affect significantly therapeutic and side effect levels.

DNA Vectorology & Gene Targeting

266. Gene Targeting at Silent Loci with Adeno-Associated Virus Vectors

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Gene targeting is a genetic engineering technique that is used to make precise modifications to the genome. In most cases, a targeting vector contains an exogenous promoter and a selection marker between two homology arms. When a target locus is silent, it can be difficult to obtain targeted clones. This could be due to a reduction in homologous recombination, and/or a suppression in exogenous promoter activity. Here we designed an assay to study gene targeting frequencies and to compare exogenous promoter activity at a silent locus in human iPSCs. The iPSCs were originally reprogrammed from mesenchymal stem cells (MSCs), after an IRES-NEO cassette had been inserted into exon 1 of the robustly expressed *COL1A1* gene. The iPSCs became G418-sensitive due to suppression of the endogenous *COL1A1* promoter driving the NEO gene. We transduced these iPSCs with a series of AAV targeting constructs and selected with G418. The AAV vectors shared the same 5' and 3' homology arms and were designed to insert different promoters between the IRES and NEO sequences in the iPSCs, such that only gene-targeted cells with an active exogenous promoter became G418-resistant. The results showed that UCOE was the best promoter, achieving a G418-resistance frequency of 0.4%, followed by PGK and EF1α promoters. AAV vectors with SOX2, REX1 and EPC promoters produced few or no G418-resistant colonies. These results suggested that promoter suppression could explain the differences between the vectors, since they all shared the same homology arms. To test this, we transduced the iPSCs with three of these AAV vectors at the same MOI and cultured the cells without selection. Gene targeting frequencies were obtained by Taqman qPCR, which measured copy numbers of