Chimerism in the DNA of buccal swabs from recipients after allogeneic hematopoietic stem cell transplantations: implications for forensic DNA testing

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Introduction

Buccal swabs are the favorite method for the collection of forensic DNA reference samples, as this approach is straightforward and non-invasive. The reliability of comparative DNA testing using buccal cells as reference is beyond discussion and is based on the fact, that the DNA is the same in every cell of the body. Therefore all exceptions undermining this basic assumption are of vital forensic importance. It has been shown, that in recipients of allogeneic hematopoietic stem cell transplantations (allo-HCT) donor-derived DNA can be found in different tissues, e.g. the buccal mucosa. A mixed chimerism in the DNA from buccal swabs can be seen in these cases. However, a systematic documentation of this phenomenon in forensics is still lacking.

category		n	%
number of recipients	total	77	100
	male	46	60
	female	31	40
number of samples	buccal swabs	162 (from 77 recipients)	
	blood	136 (from 76 recipients)	
	hair roots	88 (from 62 recipients)	
recipient/donor relationship	related	48	62
	not related	29	38
disease	acute myelogenous leukemia	29	38
	chronic myelogenous leukemia	11	14
	acute lymphoblastic leukemia	10	13
	myelodysplastic syndrome	7	9
	multiple myeloma	5	6
	secondary acute leukemia	5	6
	aplastic bone marrow	4	5
	other	6	8
myelo-ablative	yes	50	65
	no	27	35
number of transplantations	1x	67	87
	2x	7	9
	3x	3	4
origin of stem cells	peripheral blood	70	91
	bone marrow	7	9

Table 1: Summarization of sampling and clinical details of the patients.

Material and Methods

Buccal swabs, blood and hair roots were sampled over a period of 3 years (2008-2010) in the course of the routine follow-up examinations at different post transplantation intervals (PTI) from recipients of allo-HCT suffering from different malignant diseases (**Table 1**). The sampling regime included multiple sampling per individual at different PTIs. Partially 2 or 3 buccal samples were taken from different sites of the interior oral cavity at the same time.

STR analysis was carried out with the AmpFISTR Identifiler PCR amplification kit (AB, Applied Biosystems, Foster City, CA, USA) following the protocols recommended by the manufacturer. Analysis was performed on an AB3100 using GeneScan (both AB) and Genemarker HID V1.7 (SoftGenetics, Inc. State College, PA, USA).

Based on the known DNA profiles of donors (do) and recipients (re) prior transplantation the alleles appearing exclusively in one of the two individuals were identified. The percental donor chimerism levels (%Ch) were calculated from these informative STR markers according to the formula:

$$\%\text{donor chimerism per marker} = \frac{\Sigma\text{peak areas (do)}}{\Sigma\text{peak areas (do + re)}} * 100$$

As it is not possible to accurately quantify DNA mixture ratios exceeding 1:10 all chimerism levels under 10% and above 90% were assigned to 0% and 100%, respectively. Due to multiple sampling reported %Ch levels refer either to individual samples or are broken down on the basis of recipients.

Results

Buccal swabs

The chimeric state in 162 buccal swabs from 77 adult recipients of allo-HCT was determined. From each individual between 1 and 9 swabs were taken at known PTIs, ranging from 17 to 3.361 days (median 394 days). The median age of the recipients was 50 years, ranging between 19 and 74.

In the majority of the samples (74%) a mixed chimerism was found. %Ch levels turned out to be highly variable, ranging between 0 and 100%. The median %Ch value of 23% indicates that the recipient STR profile was the major component of the mixtures in most of the cases. Only 20% of the samples (n=33) showed a chimerism level exceeding 50%. The distribution of %Ch within all recipients is illustrated in **Fig 1a**. The male and female recipient subsamples showed a slight difference between both groups with a tendency to higher chimerism levels in males (**Fig 1b**). No correlation of %Ch with the age of the patient at the time of the transplantation was found (**Fig 2**). As shown in **Fig 3** there was no tendency of an increasing or decreasing %Ch between 17 days and 9 years after the transplantation. Individual recipients that were sampled more than once showed various trends of %Ch temporal development. Examples are outlined in **Fig 4**. %Ch of samples from different locations of the oral cavity taken at the same time from the same individual differed significantly displaying no consistent trend. Examples are listed in **Table 2**.

Blood samples and hair follicles

All 136 blood samples (76 patients) showed the donor genotype exclusively and in all 88 hair samples (62 patients) no indication of the donor genotype was found, all showing exclusively the STR profile of the recipient.

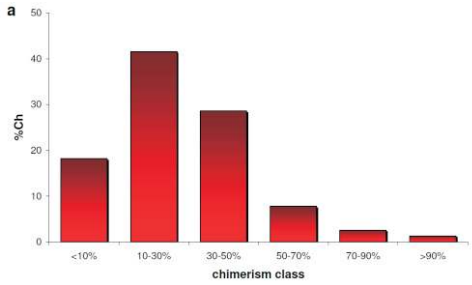

Figure 1a: Chimerism levels in buccal swabs of 77 recipients of allogeneic hematopoietic cell transplantations. The data were assigned to chimerism classes with 20% intervals except the lowest and highest class encompassing results under 10% and above 90% respectively.

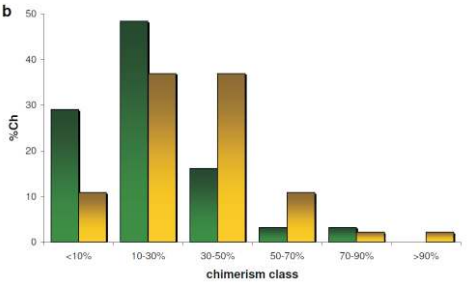

Figure 1b: Frequencies of chimerism classes in buccal swabs within the female (green) and the male (yellow) recipient subsamples.

patient Nr	PTI [days]	location	%Ch	STD
835	237	cheek side	33.1	4
		tongue	100	-
	314	cheek side	49.3	4
		tongue	100	-
890	251	cheek side 1	45	12.2
		cheek side 2	82.3	4.9
386	3361	cheek side 1	54.7	4.5
		cheek side 2	37	6.6
		lower lip	71.8	7.1

Table 2: Percental chimerism levels (%Ch) found at different locations of the oral cavity sampled simultaneously. If buccal scraping was undertaken at the left and right side this is indicated by "1" and "2". PTI – post transplantation period

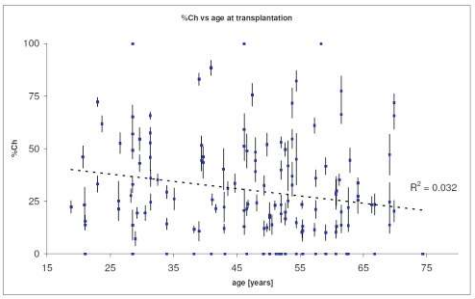

Figure 2: Percental chimerism levels of 162 buccal swabs (mean of all informative STR-markers ± STD) depending on the age of the recipients at the time of the transplantation. No correlation was found as indicated by the dotted trend line and the correlation coefficient.

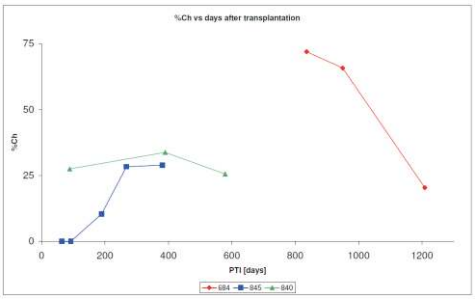

Figure 4: Time courses of %Ch for selected recipients. Recipient 840 had a constant level of app. 30%, whereas 845 showed an increase from 0% to 29%, and the %Ch of 684 decreased from 72 to 20%.

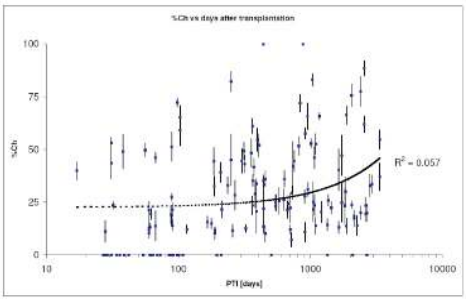

Figure 3: Percental chimerism levels of 162 buccal swabs (mean of all informative STR-markers ± STD) depending on the post transplantation interval (PTI). No correlation was found as indicated by the dotted trend line and the correlation coefficient.

- **It was shown that three sample types from one allo- HCT recipient resulted in three different STR profiles, two completely different single-source DNA profiles (blood - donor derived; hair roots - recipient derived) and one mixture (buccal swab).**
- **The occurrence of mixed chimerism in buccal swabs is the rule rather than the exception.**
- **The %Ch variability proved to be surprisingly high ranging from 0 to 100%. This applied both the variability between-individuals (Fig 1) as well as within-individuals (Fig 4 ,Tab 2).**
- **The high variability makes it impossible to deconvolute the profiles of the donor and recipient reliably without previous knowledge or additional information, e.g. recordings of medical interventions or supplemental DNA data from other tissues or from relatives.**
- **Gender, age and PTI were confirmed not to be correlated with the chimerism level (Figures 1b, 2 and 3) making %Ch levels very unpredictable.**