reprogramming process and subsequent culture of iPS cells *in vitro* can induce genetic changes. Three types of genomic abnormalities were seen: aberrations of somatic cell origin, aberrations present in early passages but not of apparent somatic cell origin, and aberrations acquired during passaging. Notably, the high incidence of chromosome 12 duplications observed by Mayshar and colleagues [69] caused significant enrichment for cell cycle-related genes, such as *NANOG* and *GDF3*. Another study reported that regions close to pluripotency-associated genes were duplicated in multiple samples [70]. Selection during hiPS cell reprogramming, colony picking and subsequent culturing may be factors contributing to the accumulation of mutations.

Impact of epigenetic differences on pluripotency

One of the goals of using hiPS cells is to generate functional target cells for medical screening and therapeutic applications. For these applications, it must be evaluated thoroughly whether small DMRs among ES and iPS cells affect the competency, differentiation propensities, stability and safety of iPS cells. It remains to be elucidated how the degree of these differences contributes to the variance in pluripotency among ES and iPS cells. Analysis of iPS cells obtained from mouse fibroblasts and hematopoietic and myogenic cells demonstrated that cellular origin influences the potential of miPS cells to differentiate into embryoid bodies and different cell types *in vitro*. In a related study, Kim and colleagues [56] compared the ability to differentiate to blood lineages of iPS cells derived from fibroblasts, neural cells, hematopoietic cells and ES cells in the mouse system, and demonstrated consistent differences in blood-forming ability - that is, blood derivatives showed more robust hematopoiesis *in vitro* than neural derivatives. Therefore, low-passage iPS cells derived from different tissues harbor residual DNA methylation signatures characteristic of their somatic tissue of origin, which favors their differentiation along lineages related to the parental cell, while restricting alternative cell fates. Similarly, Miura and colleagues [71] demonstrated that differences in gene expression in miPS cells derived from different types of parental cells result in variations in teratoma formation. These studies demonstrate that reprogramming to generate iPS cells is a gradual process that modifies epigenetic profiles beyond the acquisition of a pluripotent state.

Prediction for pluripotency and differentiation preference

Significant variation has been also observed in the differentiation efficiency of various hES cell lines [72]. Incomplete DNA methylation of somatic cells regulates the efficiency of hiPS cell generation [58], and selection of parental cell types influences the propensity for differentiation [73,74]. Such differences must be better understood before hES and hiPS cell lines can be confidently used for translational research. To predict a cell line's propensity to differentiate into the three germ layers, Bock and colleagues [52] performed DNA methylation mapping by genome-scale bisulfite sequencing and gene expression profiling using microarrays and quantified the propensity to form multiple lineages by utilizing a non-directed embryoid bodies formation assay and high-throughput transcript counting of 500 lineage marker genes in embryoid bodies using 20 hES cells lines and 12 hiPS cell lines over passages 15 to 30. They bioinformatically integrated these genomic assays into a scorecard that measures the quality and utility of any human pluripotent cell line. The resulting lineage scorecard pinpoints quantitative differences among cell-line-specific differentiation propensities. For example, one hES cell line that received a high score for endoderm differentiation performed well in directed endoderm differentiation, and other hES cell lines that received high scores for neural lineage differentiation efficiently differentiated into motor neurons. In addition, two hiPS lines that the scorecard predicted to have a low propensity to differentiate into the neural lineage were impaired in motor neuron-directed differentiation. On the other hand, other hiPS lines that the scorecard predicted to have a high propensity to differentiate into ectodermal and neural lineages were found to differentiate well into motor neurons. Therefore, the scorecard can detect lineage-specific differences in the differentiation propensities of a given cell line [52].

Functional assay for differentiated cells from iPS and ES cells

Although the propensity for differentiation could be predicted, it remains to be elucidated whether iPS cell-derived cells are functionally and molecularly the same as ES cell-derived cells. To address this issue, two studies conducted functional assays comparing differentiated neural cells derived from iPS cells to those derived from ES cells by marker gene expression and action potential measurements [75,76]. There was some variation in efficiency and quantitative differences in motor neuron generation among the lines, but the treatment of neuroepithelial cells from pluripotent stem cells with retinoic acid and sonic hedgehog resulted in the generation of iPS and ES cell lines with a neuronal morphology that expressed TUJ1. In addition, electrophysiological recordings using whole-cell patch clamping showed inward and outward currents, and it was concluded that ES cell- and iPS cell-derived neurons are similarly functional at a physiological level. These studies demonstrated that the temporal course and gene-expression pattern during

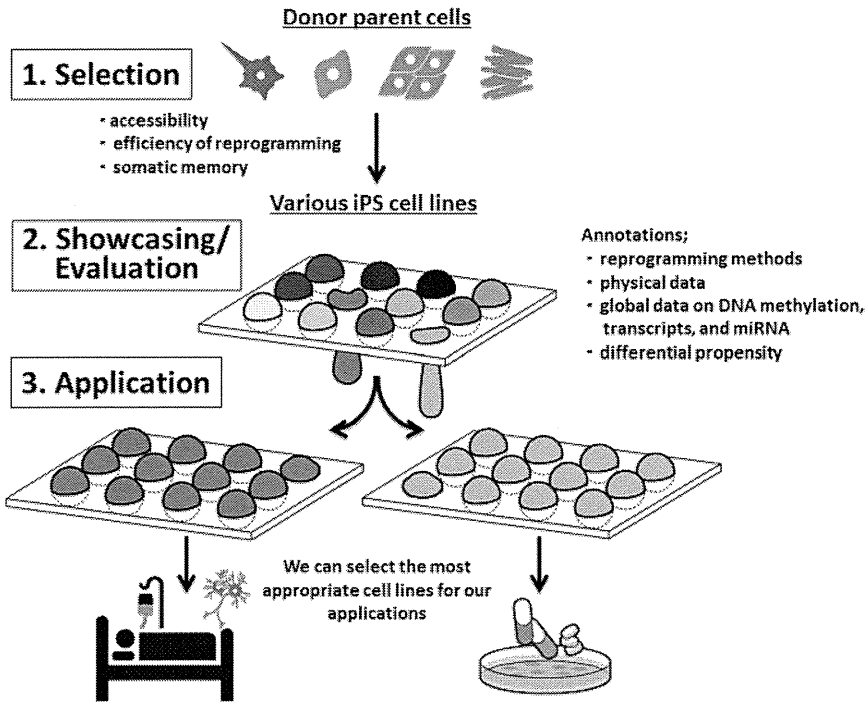

Figure 2. Workflow for human iPS cell applications. 1. Selection: choosing donor parent tissue considering accessibility, efficiency of reprogramming, and differential propensity. It would be useful to evaluate the expression of somatic memory genes, such as *C9orf64*, which reduces the efficiency of induced pluripotent stem (iPS) cell generation [58]. 2. Showcasing/evaluation: provides annotated information on reprogramming methods, culture conditions, physical data on stem cells, and global data on DNA methylation, transcription and microRNAs (miRNAs). It is very informative to integrate the genetic and epigenetic and biological data, such as differential propensity [52,76]. 3. Application: using annotation data, we can select the most appropriate iPS cell lines for our applications. Various hiPS cell lines (shown as differently shaded spheres) would be listed before further processing of the application. Valid cell lines (colored purple and blue) could be functionally and molecularly selected for appropriate applications, such as cell replacement therapy and/or drug screening.

neuroepithelial cell differentiation and production of functional neurons were nearly identical between ES and iPS cells, regardless of the reprogramming method, cellular origin, and differences between iPS and ES cells. These findings raise hopes of applying human iPS cells to the modeling of diseases and potential autologous cell transplantation.

It is important to acquire scientific information on pluripotential stem cells for further applications, such as industrial and clinical uses. Pluripotent stem cells, including disease-specific stem cells, could be showcased with useful annotation data and the most appropriate cell lines could be selected (Figure 2).

Conclusion

Many issues have yet to be resolved before the results of stem cell research can benefit the public in the form of medical treatments. In this review, we have discussed the substantial variation observed among pluripotent stem cells, including transcriptional and epigenetic profiles in the undifferentiated state, the ability to differentiate into various types of cells, and the functional and molecular nature of embryoid body or stem cell-derived differentiated cells. These results suggest that most, but not all, iPS cell lines are indistinguishable from ES cell lines, even though there is a difference between the average ES cell and the average iPS cell. Thus, ES and iPS cells should not be regarded as one or two well-defined points in the cellular space but rather as two partially overlapping point clouds with inherent variability among both ES and iPS cell lines [52,76]. Notably, human iPS cells seemed to be more variable than human ES cells. No single stem cell line may be equally powerful for deriving all cell types *in vitro*, implying that researchers would benefit from identifying the best cell lines for each application. Furthermore, for clinical use in the future, it is important to use both ES and iPS cells in research, and to standardize reprogramming methods, culture equipment and techniques and to optimize differentiation methods and evaluate the functions and tumorigenicity of differentiated cells.

This article is part of a review series on *Induced pluripotent stem cells*. Other articles in the series can be found online at http://stemcellres.com/series/ipsc

Abbreviations

DMR, differentially methylated region; ES, embryonic stem; hES, human embryonic stem; hiPS, human induced pluripotent stem; iPS, induced pluripotent stem; miPS, mouse induced pluripotent stem; miRNA, microRNA.

Competing interests

The authors declare that they have no competing interests.

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Ⅴ. 参考資料

厚生労働省発医政0822第6号
平　成　23年　8月　22日

大阪大学大学院歯学研究科
研究科長　米田　俊之　殿

厚生労働大臣　細川　律夫

ヒト幹細胞臨床研究実施計画について

平成22年10月28日付で申請のあった下記の臨床研究については、実施して差し支えない。

なお、臨床研究の中止、終了などに伴う厚生労働大臣への報告については、ヒト幹細胞を用いる臨床研究に関する指針（平成18年厚生労働省告示第425号）の定めるところによるほか、定期的に中間報告書を提出するようお願いする。

記

課　題　名：自己脂肪組織由来幹細胞を用いた新しい歯周組織再生療法開発

研究責任者：村上　伸也
（大阪大学大学院歯学研究科・教授）

様式ａ

平成２３年１１月８日

研究責任者　殿

大阪大学歯学部附属病院長
森　崎　市治郎

ヒト幹細胞臨床研究の実施に関する許可書

下記のヒト幹細胞臨床研究について、大学院歯学研究科・歯学部及び歯学部附属病院倫理審査委員会からの報告及び厚生労働大臣の意見に基づき実施を許可する。

研　究　題　目	自己脂肪組織由来幹細胞を用いた新しい歯周組織再生療法開発

添付書類
１．大阪大学大学院歯学研究科・歯学部及び歯学部附属病院倫理審査委員会審査結果通知書の写し

２．厚生労働大臣の意見書の写し

平成２３年１１月８日

村　上　伸　也　　殿

歯学研究科長
脇　坂　　聡

研　究　許　可　書

下記の臨床研究については、平成２３年８月２２日付け厚生労働省発医政０８２２第６号にて厚生労働大臣から実施して差し支えない旨意見がありましたので、研究の実施を許可します。

なお、今後、当該研究にかかわる重要な知見もしくは事情の見落としまたは出現により、許可内容通りの実施が危険ないし問題であることが明らかとなった場合には当該研究の停止、修正等の措置をとることがあり得ることを申し添えます。

記

課　題　名：自己脂肪組織由来幹細胞を用いた新しい歯周組織再生療法開発

主任研究者：口腔分子免疫制御学講座（口腔治療学教室）教授　村上　伸也

様式a

平成２４年１月１１日

研究責任者　殿

大阪大学歯学部附属病院長
森　崎　市治郎

ヒト幹細胞臨床研究の計画変更に関する許可書

下記のヒト幹細胞臨床研究について、大学院歯学研究科・歯学部及び歯学部附属病院倫理審査委員会からの報告に基づき計画変更を許可する。

研　究　題　目	自己脂肪組織由来幹細胞を用いた新しい歯周組織再生療法開発

添付書類
・大阪大学大学院歯学研究科・歯学部及び歯学部附属病院倫理審査委員会審査結果通知書の写し

平成２４年１月１１日

村　上　伸　也　　殿

歯学研究科長
脇　坂　　聡

研　究　変　更　許　可　書

貴殿より変更申請のありました下記の課題について、研究内容を変更することを承認しましたので通知します。

なお、今後、当該研究にかかわる重要な知見もしくは事情の見落としまたは出現により、承認内容通りの実施が危険ないし問題であることが明らかとなった場合には実験の停止、修正等の措置をとることがあり得ることを申し添えます。

記

受　付　番　号	Ｈ２１－Ｅ３０－１
課　　題　　名	自己脂肪組織由来幹細胞を用いた新しい歯周組織再生療法開発
主任研究者所属	口腔分子免疫制御学講座（口腔治療学教室）
主任研究者氏名	村上　伸也

ヒト幹細胞臨床研究実施計画書

「自己脂肪組織由来幹細胞を用いた

新しい歯周組織再生療法開発」

研究責任者：村上伸也
研究分担者（主任）：北村正博
所属機関名：大阪大学大学院歯学研究科
所属科・部署：口腔治療・歯周科

第一版：作成年月日 2011 年 11 月 8 日

大阪大学歯学部附属病院病院長 森岡宗治郎 日付 平成23年11月8日

改訂履歴表

改訂番号	年月日	改訂内容	改訂理由	承認
00	H23.11.8	制定		

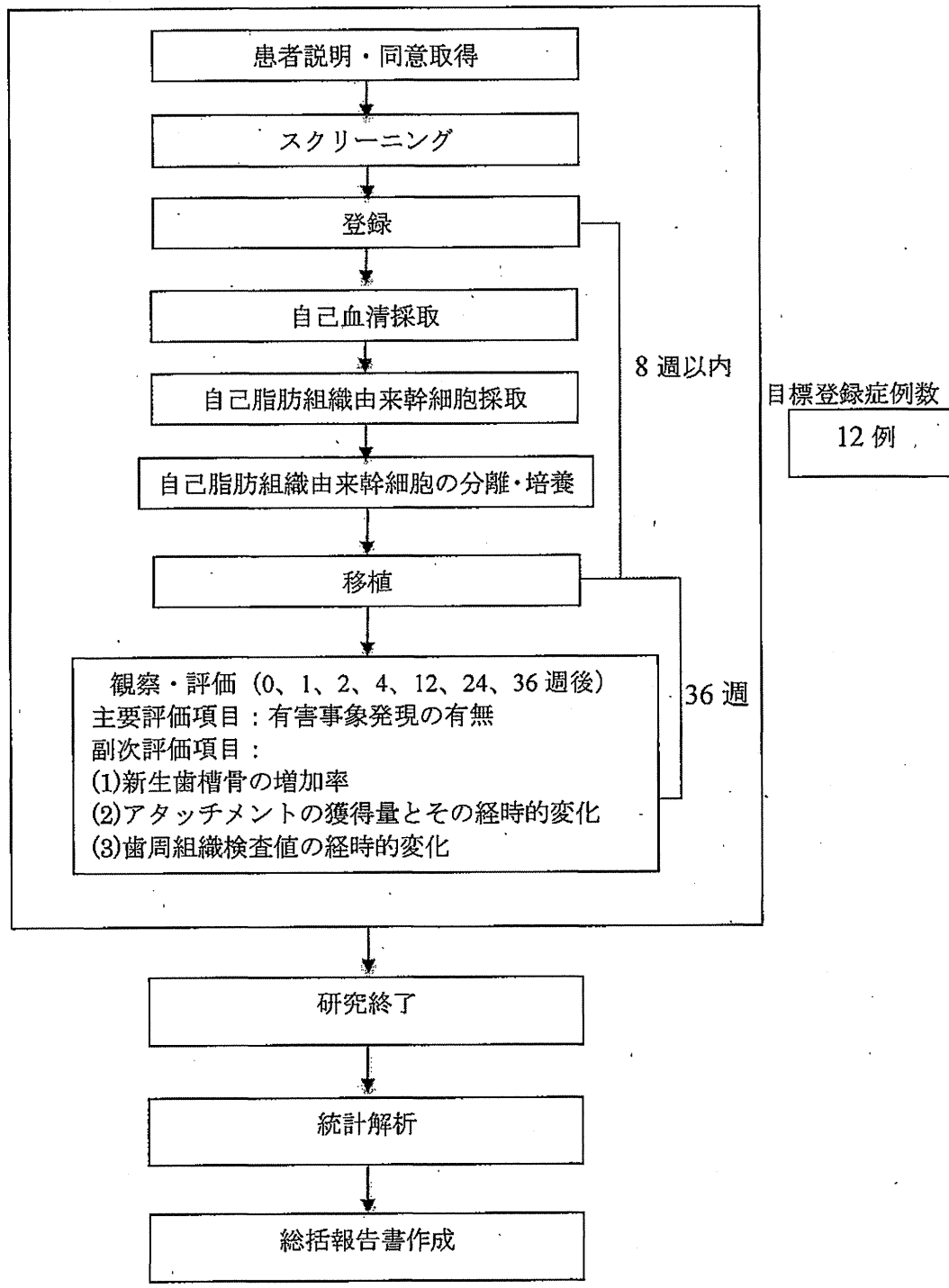
概要

項目	内容
目的	辺縁性歯周炎患者を対象として、フラップ手術を施行する際に、自己脂肪組織由来幹細胞を移植し、自己脂肪組織由来幹細胞移植術に基づく歯周組織再生療法の安全性、有効性及び実施可能性を評価することを目的とした。
対象疾患	従来の治療法では十分な歯周組織欠損の回復が見込めない辺縁性歯周炎
選択基準	1) 初診時にプロービングデプス 7mm 以上の歯周ポケットが認められる患者。 2) X 線写真により、深さ 4mm 以上かつ幅 2mm 以上の垂直性骨欠損が歯間部（被験歯の近心または遠心のいずれかを含む位置）に認められる患者。 3) 被験者の選択に至る再評価において、初期治療内容が達成されている患者。 4) 被験歯の動揺度が 2 度以下で、かつフラップ手術が適応と判断される角化歯肉が存在する患者。 5) 口腔衛生が確立しており、幹細胞移植術後も研究責任者又は分担者の指導に従った口腔清掃を行うことが可能であると研究責任者又は分担者が判断した患者。 6) 同意取得時に 20 歳以上の男女。 7) 本臨床研究の参加について文書により同意が得られている患者。
除外基準	1) 臨床的アタッチメントレベルの正確な測定に支障をきたす補綴物等が存在している患者。 2) 悪性腫瘍を合併している、またはその既往がある患者。 3) 登録前口腔内診断において、口腔内に悪性腫瘍、前癌病変またはそれらが疑われる所見のある患者。 4) ビスホスホネート系薬剤を使用したことのある患者、使用する予定のある患者。 5) 幹細胞移植術後 36 週以内に被験部位の評価に影響を及ぼす処置（外科的処置または被験歯の補綴処置や根管処置等）を行うことが必要な患者。 6) 妊娠中、授乳中、移植 36 週後までに妊娠を希望している、または妊娠の可能性がある患者（登録前妊娠検査により判断）。 7) 腎障害、肝障害、血液障害を合併している患者。 8) 登録前臨床検査でヘモグロビン A1c が 6.5%以上の患者。 9) 登録前臨床検査でヘモグロビンが 6.0g/dL 未満又は血小板が 5.0×10^4/mL 未満の患者。 10) 活動性の感染症を有する患者。 11) 登録前 6 ヵ月以内にアルコール中毒症又は薬物依存症の既往を有する患者。 12) 精神疾患を合併、又は精神疾患の症状を呈している患者。 13) HCV 抗体、HBs 抗原、ATLA、HIV 抗体陽性の患者。 14) その他、研究責任者の判断により、当研究への参加が不適当と考えられる患者。
被験者の同意	スクリーニングを行う前に外来において同意説明を行い、被験

	者本人による同意を得る。 研究責任者又は分担者は、本研究への参加候補となる被験者本人に対して、同意説明文書（添付文書「患者さんへ」参照）を提供し、口頭で十分な説明を行った後、本研究への参加の同意を文書で取得する。なお、本研究においては、単独で同意を取得できない者は被験者としない。
試験物	自己脂肪組織由来幹細胞
研究方法 (治療スケジュール)	1）自己血清の採取 自己脂肪組織採取前30日以内に大阪大学医学部附属病院輸血部において自己血採取バッグに400mLの血液を採取・遠心分離し、血清成分を大阪大学歯学部附属病院CPC（Cell Processing Center）において凍結保存する。 2）自己脂肪組織の採取 登録完了後（「13. 登録」参照）、手術室において、局所麻酔下にて腹部脂肪採取部位にメスで1cm程度の切り口を開け、カニューレを挿入する。シリンジを引き陰圧の状態にして固定し、皮下に針を巡らしながら脂肪組織を吸引する。吸引した脂肪組織は直ちに滅菌検査用コップに移し替える。必要量の脂肪組織10~20gが採取できるまでこの操作を1~数回繰り返す。脂肪採取終了後、切開部の消毒・縫合を行い、テガダームを貼付し、必要に応じレストンスポンジにて圧迫する。疼痛および感染症の予防のため鎮痛剤および抗生物質の内服投与を行う。 なお、腹部から十分な脂肪組織が採取できない場合は、大腿からの採取を追加する。 3）自己脂肪組織からの幹細胞の単離および培養 採取した脂肪組織を保存溶液に浸漬し、大阪大学歯学部附属病院 Cell Processing Center にて幹細胞を単離し、移植細胞数に達するまで、1~2 週間程度の継代培養を行い凍結する。なお、規格を満たさない場合には、再度採取工程を実施する。2 回実施しても規格を満たさない場合には被験者の臨床研究を中止する。凍結した幹細胞は、移植術の 3 日前に解凍し、移植当日まで培養する。 4）培養自己脂肪組織由来幹細胞移植術 継代培養した自己脂肪組織由来幹細胞の回収し、試験物 200μL（フィブリンゲルに懸濁した培養自己脂肪組織由来幹細胞 3.0×10^6 個/200μL）に調製、出荷判定結果を確認した後、研究責任者又は分担者は、移植術を施行する。その際、フィブリン懸濁物を患部歯槽骨欠損部の形態に合わせてスパーテルを用いて填入、移植する。
併用禁止薬剤及び 併用禁止療法	ビスホスホネート系薬剤は臨床研究終了まで使用しないこととする。 被験部位（被験歯及び隣接歯）に対して評価に影響を及ぼす処置（外科的処置または被験歯の補綴処置や根管処置等）は、臨床研究終了まで行わないこととする。
観察・検査スケジュールの概略	観察・検査スケジュール表参照
主要評価項目	安全性の確認

目標登録症例数	12　例
研究登録期間	大阪大学歯学部附属病院長による実施の許可（平成 23 年 11 月 8 日）から 2 年間

シェーマ


患者説明・同意取得
スクリーニング
登録
自己血清採取
自己脂肪組織由来幹細胞採取
自己脂肪組織由来幹細胞の分離・培養
移植
観察・評価（0、1、2、4、12、24、36 週後）
主要評価項目：有害事象発現の有無
副次評価項目：
(1)新生歯槽骨の増加率
(2)アタッチメントの獲得量とその経時的変化
(3)歯周組織検査値の経時的変化
8 週以内
36 週
目標登録症例数
12 例
研究終了
統計解析
総括報告書作成

観察・検査スケジュール

以下のスケジュール表に従って、観察・検査・評価を実施する。

観察・評価日		前観察※2	0日	1週後	2週後	4週後	12週後	24週後	36週後	中止時
許容範囲		90日以内	移植日	±3日		±1週	±2週			
全身所見		○	○	○	○	○	○	○	○	○
口腔内所見		○	○	○	○	○	○	○	○	○
脂肪組織採取部位所見		○※1	○			○	○		○	○
臨床検査	血液	○	○	○		○	○		○	○
	尿	○	○	○		○	○		○	○
	十二誘導心電図	○				○			○	○
画像診断	胸部X線検査	○				○			○	○
	局所X線写真撮影	○				○	○	○	○	○
歯周組織検査	臨床的アタッチメントレベル歯周組織検査	○	○				○	○	○	○

○：被験者の状態により検査をおこなうこと自体に危険が伴うと判断されたときを除いて、実施許容期間内に観察、検査、評価を必ず実施する。

※1：脂肪組織採取1週後（±3日）に行う。

※2：前観察項目は手術の90日前以内のものであれば登録前のスクリーニング検査で代用することができる。

語句の定義

フラップ手術：中等度以上に進行した歯周炎の処置として、歯肉を切開・剥離して歯周疾患に罹患した病変部を掻爬し、歯石や壊死セメント質を除去して滑沢となった歯根面と歯肉弁を再付着させる手術法。

プローブ：歯周ポケットの測定に用いる器具。

プロービング：歯周ポケット探査。歯周疾患の治療時、歯周組織の診査をする際に、歯周ポケット内、歯根表面、歯間隣接面部をプローブで探査すること、歯周ポケットの深さ・形態、歯肉炎症の広がり程度、根分岐部の形態、歯石・プラークの付着状態（量、付着範囲）、歯槽骨の形態・位置などの診査を行う。

臨床的アタッチメントレベル（CAL）：歯周疾患の進行と治癒の程度を知るための診査項目であり、ポケット底の付着部位が歯面のどこに位置するのかを示す一つの指標。アタッチメントレベルはプローブを使用し、エナメルセメント境を基準としてそこからポケット底までの距離（mm）を計測した値で表す。

参考文献：歯科医学事典（医歯薬出版株式会社）

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